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listing and declared, that it includes no new matter. The applicant has subsequently filed a sequence

<u>54</u> Human G-protein coupled receptor protein cloned form fetal brain CDNA library

a method for screening for compounds which inhibit ligprotein, determination of a ligand to the receptor protein, coding the receptor protein, production of the receptor partial peptide and their salts are disclosed. DNA en-A novel G-protein coupled receptor protein, a

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for such compounds are also disclosed. The receptor screening for candidate compounds of drugs and the protein, its partial peptide and their salts are used for and binding to the receptor protein, a kit for screening



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Description

FIELD OF THE INVENTION

The present invention relates to novel G-protein coupled receptor proteins derived from a human brain, or salts

BACKGROUND OF THE INVENTION

- guanine nucleotide-binding proteins (hereinafter, sometimes, referred to as G-proteins) and are generally called Gin a cell membrane. Many of these receptor proteins mediate signal transmission in a cell by activation of coupled naving seven transmembrane domains. protein coupled receptor proteins or 7-transmembrane receptor proteins because they contain a common structure Many hormones and neurotransmitters regulate functions in a living body through specific receptor proteins existing
- the living body, for example, hormones, neurotransmitters, physiologically active substances and the like. body and play very important roles as targets of molecules which regulate functions of the cells and internal organs of G-protein coupled receptor proteins exist on each functional cell surface of cells and internal organs of a living
- 8 in numerous sites within a brine and regulate the physiological functions thought their corresponding receptor proteins organ such as a brain, its physiological functions are controlled through regulation by many hormones, hormone-like for development of drugs having close relation to such functional mechanisms. For example, in a central nerve system portant means for clarification of functional mechanisms of cells and internal organs of various living body as well as living bodies and their specific receptor proteins, in particular, G-protein coupled receptor proteins provide a very imsubstances, neurotransmitters, physiologically active substances or the like. In particular, neurotransmitters are found To clarify the relation between substances which regulate elaborate functions in cells and internal organs of various
- are subtypes of known receptor proteins. many structures of cDNAs encoding such proteins have not yet been reported. In addition, it is still unknown if there However, it is supposed that many unknown neurotransmitters still exist in a brain and, as for their receptor proteins
- receptor protein genes expressed in a brain and to express them in a suitable expression system. antagonists to receptor proteins, efficiently, in development of drugs, it is required to clarify functional mechanisms of receptor proteins provide a very important means for development of drugs. Further, for screening for agonists and Also, to clarify the relation between substances which regulate elaborate functions in a brain and their specific

Recently, as a means for analyzing genes expressed in a living body, random analysis of cDNA sequences has

딿 5) and No. T27053 (SEQ ID NO: 6), have been registered with the data base, NCBI dbEST, their functions are not functions from their sequential information only. For example, although two ESTs, accession No. T08099 (SEQ ID NO Expressed Sequence Tags (ESTs) and are publicly available. However, for many of ESTs, it is difficult to deduce their been studied actively. The sequences of cDNA fragments thus obtained have been registered with data bases as

OBJECTS OF THE INVENTION

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- brain, their partial peptides and their salts. One object of the present invention is to provide novel G-protein coupled receptor proteins derived from a human
- receptor proteins or their partial peptides. Another object of the present invention is to provide isolated DNA comprising DNA encoding the G-protein coupled
- ŝ having the recombinant vector. A further object of the present invention is to provide a recombinant vector comprising the DNA and a transformant
- Still another object of the present invention is to provide a method for determining ligands to the G-protein coupled A further object of the present invention is to provide a process for preparing the G-protein coupled receptor proteins

- to the G-protein coupled receptor proteins, a kit for screening for the receptor-agonists or antagonists, the receptor receptor-agonists or antagonists. agonists or antagonists obtained by the screening and a pharmaceutical composition comprising at least one of the Still another object of the present invention is to provide a method for screening for receptor-agonists or antagonists
- ន their partial peptides or salts. Yet another object of the present invention is to provide antibodies against the G-protein coupled receptor proteins
- skilled in the art from the following description with reference to the accompanying drawings. These object as well as other objects and advantages of the present invention will become apparent to those

BRIEF EXPLANATION OF DRAWINGS

Fig. 1 is a nucleotide sequence encoding the human G-protein coupled receptor protein (short form) of the present invention obtained in Example 1 hereinalter and its amino acid sequence deduced from the nucleotide sequence.

Fig. 2 is a graph illustrating hydrophobic plotting of the human G-protein coupled receptor protein (short form) of the present invention prepared based on the amino acid sequence of Fig. 1. The parts represented by 1 to 7 are hydrophobic domains.

Fig. 3 is a nucleotide sequence encoding the human G-protein coupled receptor protein (long form) of the present

Invention obtained in Example 1 hereinafter and its amino acid sequence deduced from the nucleotide sequence. Fig. 4 is a graph illustrating hydrôphobic plotting of the human G-protein coupled receptor protein (long form) of the present invention prepared based on the amino acid sequence of Fig. 1. The parts represented by 1 to 7 are hydrophobic domains.

Fig. 5 illustrates the results of northan hybridization for examining expression levels of mRNA encoding the human G-protein coupled receptor protein of the present invention in various human tissues. The value (kb) represents the size of the RNA molecular weight marker.

SUMMARY OF THE INVENTION

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of G-protein coupled receptor proteins derived from human fetal brain and human adult brain based on two kinds of publicly available EST information registered with a data base whose functions are unknown, and have succeeded in analysis of the entire nucleotide sequences. When their amino acid sequences have been deduced from the nucleotide sequences, the first to the seventh transmembrane domains have been confirmed on hydrophobic plotting. Thus, the As a result of an intensive study, the present inventors have succeeded in isolation of cDNAs encoding two kinds proteins encoded by these cDNAs have been confirmed to be 7-transmembrane type G-protein coupled receptor protains. The present invention has been completed based on these findings. 8 52

That is, according to the present invention, there are provided:

(1) A G-protein coupled receptor protein which comprises the same or substantially the same amino acid sequence as that represented by SEQ ID NO: 1, or its salt;

The G-protein coupled receptor protein of the above (1) which comprises the same or substantially the same <u>N</u>

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(3) A partial peptide of the G-protein coupled receptor protein of the above (1) or its salt; amino acid sequence as that represented by SEQ ID NO: 2, or its salt;

(4) An isolated DNA comprising DNA having a nucleotide sequence encoding the G-protein coupled receptor

protein of the above (1);

(5) The isolated DNA of the above (4) having the nucleotide sequence represented by SEQ ID NO: 3; (6) The isolated DNA of the above (4) having the nucleotide sequence represented by SEQ ID NO: 4;

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(7) A recombinant vector comprising the DNA of the above (4);

(8) A transformant comprising the recombinant vector of the above (7);

(9) A process for preparing the G-protein coupled receptor protein of the above (1) or its salt which comprises cultivating the transformant of the above (8) to form the G-protein coupled receptor protein; ŝ

(10) A method for determining a ligand to the G-protein coupled receptor protein of the above (1) or its salt which comprises bringing the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its salt into contact with a test compound;

(11) A method for screening for compounds which atter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises comparing (i) ligand binding upon bringing the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its salt into contact with the ligand, and (ii) that upon bringing the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its salt into contact with the ligand and a test compound;

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(12) A kit for screening for compounds which after binding of a figand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises as an essential component the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its salt;

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(13) The compounds which after ligand binding to the G-protein coupled receptor protein of the above (1) or its salt obtained by the screening method of the above (11) or the kit of the above (12), or their salts; and

(14) An antibody against the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide

More specifically, the present invention provides:

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I having a delation of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino preferably about 1 to about 30, more preferably about 1 to about 10 amino acids, or a variant of the amino acid 15) The G-prolein coupled receptor protein of the above (1) or its salt, wherein the protein comprises the amino acid sequence represented by SEQ ID NO: 1, a variant of the amino acid sequence represented by SEQ ID NO: acids, a variant of the amino acid sequence represented by SEQ ID NO: 1 having an addition of one or more, sequence represented by SEQ ID NO: 1 having a substitution of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids;

2 having a daletion of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids, a variant of the amino acid sequence represented by SEQ ID NO: 2 having an addition of one or more, (16) The G-protein coupled receptor protein of the above (2) or its salt, wherein the protein comprises the amino acid sequence represented by SEQ ID NO: 2, a variant of the amino acid sequence represented by SEQ ID NO: preferably about 1 to about 30, more preferably about 1 to about 10 amino acids, or a variant of the amino acid sequence represented by SEQ ID NO: 2 having a substitution of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids;

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(17) The method for determining a figand of the above (10), wherein the ligand is angiotensin, bombesin, cannabnoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PAC-AP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal and related polypeptides), dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related proteins), leukotriene, pancreastacin, prostaglandin, thromboxane, adenosine, adrenalin, α or β-chemokine (e.g., IL-8, GROa, GROB, GROY, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-13, MIP-13, RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptides or gallamine;

(18) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein or its salt of the above (1), or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount of the labeled ligand bound to the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its saft upon bringing the protein of the above (1), the partial peptide of the above (3) or a salt thereof into contact with the labeled ligand, and (ii) that upon bringing the protein of the above (1), the partial peptide of the above (3) or a salt thereof into contact with the labeled ligand and a test compound;

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(19) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount of the labeled ligand bound to cells containing the G-protein coupled receptor protein of the above (1) upon bringing the labeled ligand into contact with the cells with (ii) that upon bringing the labeled ligand and a test compound into contact with the cells;

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(20) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount of the labeled ligand bound to a membrane fraction of cells containing the G-protein coupled receptor protein of the above (1) upon bringing the labeled ligand into contact with the cell membrane fraction, and (ii) that upon bringing the labeled ligand and a test compound into contact with the cell membrane fraction;

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(21) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount of the labeled ligand bound to the G-protein coupled receptor protein expressed on the cell membrane of the transformant of the above (8) by cultivating the transformant upon bringing the tabeled ligand into contact with the expressed G-protein coupled receptor protein, and (ii) that upon bringing the labeled ligand and a test compound into contact with the expressed G-protein coupled receptor protein;

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(22) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises measuring and comparing (i) a cell stimulation activity mediated by the G-protein coupled receptor protein upon bringing a compound which activates the G-protein coupled receptor protein of the above (1) or its salt into contact with cells containing the G-protein coupled receptor protein of the above (1), and (ii) that upon bringing the compound which activates the G-protein coupled receptor protein or its salt and a test compound into contact with the cells;

(23) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises measuring and comparing (i) a cell stimulation activity mediated by the G-protein coupled receptor protein upon bringing a compound which activates the G-protein coupled receptor protein of the above (1) or its salt into contact with the G-protein coupled receptor protein expressed on the cell membrane of the transformant of the above (8) by cultivating the transformant, and (ii) that upon bringing the compound which activates the G-protein coupled receptor protein or its salt and a test compound into contact with the G-protein coupled receptor protein expressed on the cell membrane;

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(24) The method for screening of the above (22) or (23), wherein the compound which activates the G-protein coupled receptor protein of the above (1) is angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, se-

ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.), endothelin, enteroprostaglandin, thromboxane, adenosine, adrenalin, α or β-chemokine (e.g., IL-8, GROα, GROβ, GROγ, NAP-2, ides), dopamine, motilin, arrylin, bradykinin, CGRP (calcitonin gene related proteins), leukotriene, pancreastacin adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vascactive intestinal and related polypepgastrin, histamine, neurotensin, TRH, pancreatic polypeptides or gallamine rotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin,

(25) Compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt obtained in any one of the method of screening of the above (11) and (18) or (24), or their salts;

(27) The screening kit of the above (12) comprising cells containing the G-protein coupled receptor protein of the (26) A pharmaceutical composition comprising the compound of the above (25) or its salt;

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(28) The screening kit of the above (12) comprising a membrane fraction of cells containing the G-protein coupled receptor protein of the above (1);

obtained by using the screening kit of the above (12), (27) or (28), or its salt, (29) The compound which alter ligand binding to the G-protein coupled receptor protein of the above (1) or its salt

G-protein coupled receptor protein of the above (1), the partial peptide of the above (3) or a salt thereof. peptide of the above (3) or its salt which comprises bringing the antibody of the above (14) into contact with the (31) A method for determining the G-protein coupled receptor protein of the above (1), or its salt or the partial (30) A pharmaceutical composition comprising the compound of the above (29) or its salt; and

DETAILED DESCRIPTION OF THE INVENTION

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by SEQ ID NO: 1 (Fig. 1) and it may be the receptor protein having the same or substantially the same amino acid sequence as that represented by SEQ ID NO: 2 (Fig. 3). The amino acid sequence of SEQ ID NO: 2 is a variant of the protein") is the receptor protein which has the same or substantially the same amino acid sequence as that represented amino acid sequence represented by SEQ ID NO: 1 having an addition of 61 amino acids at the N-terminal end of SEQ The G-protein coupled receptor protein of the present invention (hereinafter sometimes abbreviated to *receptor

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မွ ષ્ઠ â another mammal (e.g., guinea pig, rat, mouse, chicken, rabbit, pig, sheep, cattle, monkey, etc.), for example, splenic cancer cells thereof and the like; and any tissues containing such cells, for example, brain, various parts of brain (e cell, nerve cell, glia cell, β cell of pancreas, marrow cell, mesangial cell, Langerhans' cell, epidermic cell, epithelial cell, olfactory bulb, amygdala, cerebral basal ganglia, hippocampus, thalamus, hypothalamus, substhanlamic nucleus osteocyte, osteoblast, osteoclast, mammary gland cell, hepatocyte, or interstitial cells or precursor cells, stem cells or cell, mast cell, neutrophil, basophil, eosinophilic leukocyte, monocyte, etc.), megakaryocyte, synovial cell, chondrocyte, endothelial cell, fibroblast, fibrocyte, muscular cell, fat cell, immunocyte (e.g., macrophage, T cell, B cell, natural killer ÖNÖ. peripheral blood, peripheral blood leukocyte, intestinal tract, prostate, testicle, testis, ovarium, placenta, uterus, bone marrow, adrenal gland, skin, muscle, lung, digestive tract, blood vessel, heart, thymus, spleen, submandibular gland stantia nigra), spinal cord, pituitary, stomach, pancreas, kidney, liver, genital organs, thyroid gland, galibladder, bone cerebral cortex, medulta, cerebellum, occipital pole, frontal lobe, pulamen, caudate nucleus, corpus callosum, subint, small intestine, large intestine, skeletal muscle and the like, in particular, brain and various parts of brain. And The receptor protein of the present invention may be any peptide derived from any cells of a human being and

Ġ or SEQ ID NO: 2 and having substantially the same activity as that of the receptor prolain comprising the amino acid homology, more preferably at least about 90% homology to the amino acid sequence represented by SEQ ID NO: 1 1 or SEQ ID NO: 2" includes any protein which has at least about 70% homology, preferably at least about 80% The wording "the same or substantially the same as the amino acid sequence as that represented by SEQ ID NO peptide may be a synthetic one.

Therefore, quantitative factors such as degrees of ligand binding activity and signal information transmission activity and the like. The wording "substantially the same" means that the natures of their activities are equal to one another sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2. Examples of substantially the same activity include ligand binding activity, signal information transmission activity

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an addition of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids, or a sequence represented by SEQ ID NO: 2 having a deletion of one or more, preferably about 1 to about 30, more may differ from one another. about 1 to about 30, more preferably about 1 to about 10 amino acids. variant of the amino acid sequence represented by SEQ ID NO: 2 having a substitution of one or more, preferably preferably about 1 to about 10 amino acids, a variant of the amino acid sequence represented by SEQ ID NO: 2 having Further, the receptor protein of the present invention may be a protein comprising a variant of the amino acid

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More specifically, the receptor protein of the present invention includes, for example, the receptor protein compris

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receptor protein comprising the amino acid sequence represented by SEQ ID: NO 2 derived from a human brain. ing the amino acid sequence represented by SEQ ID: NO 1 derived from a human brain, or the G-protein coupled

õ of an amino acid in the molecule of the above receptor protein is protected with a suitable protecting group (e.g., acyl a substituent (e.g., -OH, -COOH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chain region of the above receptor protein is cleaved in a living body and the glutarnyl group formed is pyroglutaminated; or a protecting group (e.g., acyl group having 1 to 6 carbon atoms such as formyl group, acetyl group, etc.); the N4erminal protein, wherein the amino group of the N-terminal methionine residue of the above receptor protein is protected with group having 1 to 6 carbon atoms such as formyl group, acelyl group, etc.), or conjugated proteins of the above receptor protein such as glycoproteins having sugar chains Furthermore, examples of the receptor protein of the present invention include variants of the above receptor

š lett hand end (amino terminal) is the N-terminal and the right hand end (carboxyl terminal) is the C-terminal. And, in 6 to 12 carbon atoms such as phenyl, α -naphthyl, etc., an aralkyl having 7 to 14 carbon atoms such as a phenyl- $C_{1.2}$ butyl, etc., a cycloalkyl group having 3 to 8 carbon atoms such as cyclopentyl, cyclohexyl, etc., an aryl group having R of the ester group include an alkyl group having 1 to 6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-(-COOH) or carboxylate (-COO), but the C-terminal may be the arnide (-CONH₂) or an ester (-COOH). Examples of the amino acid sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2, normally, the C-terminal is carboxyl group the receptor protein of the present invention, a representative example thereof being the receptor protein comprising alkyl group (e.g., benzyl, phenethyl, etc.), an α-naphthyl-C₁₋₂ alkyl group (e.g., α-naphthylmethyl, etc.) and the like. In The receptor protein of the present invention is represented by a conventional manner in peptide art. That is, the

the C-terminal, it may be amidated or esterified and such amide or ester is also included in the scope of the receptor addition, pivaloyloxymethyl ester or the like which is used widely as an ester for oral administration can also be used. protein of the present invention. The ester group may be the same group as that described with respect to the above When the receptor protein of the present invention has a carboxyl group (or carboxylate) at a position other than

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self is preferred. Examples of the salt include those with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrochromic acid, sulfuric acid, enc.) and those with organic acids (e.g., acetic acid, formic acid, propionic acid, furnaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesultonic acid, benznesuttonic acid, etc.). As the salt of the receptor protein of the present invention, in particular, a physiologically acceptable acid addition

မ of a human being or another mammal by a per se known purification method of proteins. Alternatively, the receptor receptor protein of the present invention or according to a peptide synthesis method as described hereinafter. protein or its salt of the present invention can be prepared by cultivating a transformant containing DNA encoding the The receptor protein or its salt of the present invention can be prepared from the above-described cells and tissues

ક્ષ and then extracted with, for example, an acid. The extract can be purified and isolated by combining chromatographies such as reverse phase chromatography, ion exchange chromatography and the like. When it is produced from cells or tissues of a human being or another mammal, the cells or tissues are homogenized

đ invention, for example, a part of the receptor protein molecule of the present invention which is exposed to outside of 4. A peptide containing a hydrophobic domain part can be similarly used. In addition, the peptide may contain each been analyzed to be extracellular domains (hydrophilic domains) in the hydrophobic plotting analysis as shown by Fig. receptor protein having the amino acid sequence represented by SEQ ID NO: 2 is that containing the parts which have domains (hydrophilic domains) in the hydrophobic plotting analysis as shown by Fig. 2. The partial peptide of the sequence represented by SEQ ID NO: 1 is a peptide containing the parts which have been analyzed to be extracellular a cell membrane or the like can be used. Specifically, the partial peptide of the receptor protein having the amino acid As the partial peptide of the receptor protein (hereinafter sometimes abbreviated to "partial peptide") of the present

amino acids, the 254th to 265th amino acids, the 335th to 368th and the 448th to 459th amino acids of the amino acid the 193rd to 204th amino acids, the 274th to the 307th amino acids, and the 387th to 398th amino acids of the amino domain separately or plural domains together. acid sequence represented by SEQ ID NO: 1 as well as those having the amino acid sequences of the 139th to 191st Examples of the partial peptide include those having the amino acid sequences of the 78th to 130th amino acids

protecting group (e.g., acyl group having 1 to 6 carbon atoms such as formyl group, acetyl group, etc.); the N4erminal peptide, wherein the amino group of N-terminal methionine residue of the above receptor protein is protected with a sequence represented by SEQ ID NO: 2. region of the above receptor protein is cleaved in a living body and the glutarnyl group formed is pyroglutaminated; or Further, the partial peptide of the receptor protein of the present invention include variants of the above partial

a substituent (e.g., -OH, -COOH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chain of an amino acid in the molecule of the above receptor protein is protected with a sultable protecting group (e.g., acy group having 1 to 6 carbon atoms such as formyl group, acetyl group, etc.), or conjugated peptides of the above partial peptide such as glycopeptides having sugar chains.

Normally, the C-terminal of the partial peptide of the present invention is a carboxyl group (-COOH) or carboxylate (-COO') and, like the receptor protein of the present invention, the C-terminal may be the amide or ester. When the it may be amidated or esterified and such amide or ester is also include in the scope of the partial pepide of the present partial peptide of the present invention has a carboxyl group (or carboxylate) at a position other than the C-terminal, invention. The ester group may be the same group as that described with respect to the above C-terminal of the receptor

salt is proferred. Examples of the salt include those with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrochromic acid, sulfuric acid, etc.) and those with organic acids (e.g., acetic acid, formic acid, furnanic As the salt of the partial peptide of the present invention, in particular, a physiologically acceptable acid addition acid, maleic acid, succinic acid, lartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, mathanesulfonic acid, benznesulfonic acid, etc.).

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The partial peptide or its salt of the present invention can be prepared according to a per se known peptide synthesis method or by cleaving the receptor protein of the present invention with a sultable peptidase.

As the peptide synthesis method, for example, any of solid phase synthesis and liquid phase synthesis can be employed. That is, the objective peptide can be produced by condensing a partial peptide or amino acid sequence which can compose of the partial peptide of the present invention with the remaining part and deprotecting a protecting Schroeder and Luebke, The Peptide, Academic Press, New York (1965); Nobuo Izumi et al., Fundamental and Expergroup, if any. Conventional condensing methods and deprotecting methods can be employed and they are described for example, M. Bodanszky and M.A. Ondetti, Peptide Synthesis, Interscience Publishers, New York (1966); iment of Peptide Synthesis, Maruzen (1975); Haruaki Yazima and Syunpei Skakibara, Biochemistry Experiment Lecture, Protein Chemistry IV, 205 (1977); Haruaki Yazima, Second Series Drug Development Vol. 14, Peptide Synthesis, Hirokawa Shoten.

After completion of the reaction, the partial peptide of the present invention can be purified and isolated by cominto its appropriate salt according a known method. On the other hand, the peptide obtained is in the form of a salt, it bining conventional purification methods such as solvent extraction, distillation, column chromatography, liquid chromatography, recrystallization and the like. In case the partial peptide thus obtained is a free peptide, it can be converted can be converted into the corresponding free peptide.

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of genomic DNA, genomic DNA library, cDNA derived from the above-described cells and tissues, cDNA library derived from the above-described cells and tissues and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid, phagemid and the like, in addition, the DNA can be amplified by reverse transcriptase The DNA encoding the receptor protein of the present invention may be any DNA in so far as it contains the nucleotide sequence encoding the above-described receptor protein of the present invention. The DNA may be any polymerase chein reaction (hereinalter abbreviated to RT-PCR) with a mRNA fraction prepared from the above-described cells and tissues.

Specifically, the DNA encoding the receptor protein having the same or substantially the same amino acid sequence represented by SEQ ID NO: 1 of the present invention may be, for example, DNA having the nucleotide sequence represented by SEQ ID NO: 3 or any DNA having a nucleotide sequence hybridizable to the nucleotide sequence represented by SEQ ID NO: 3 under high stringent conditions and encoding a receptor protein which has the same activities, i.e., ligand binding activity, signal information transmission activity and the like as those of the receptor protein DNA having at least about 70% to about 80% homology, preferably, at least about 90% homology, more preferably, at peptide having the amino acid sequence represented by SEQ ID NO: 1. Examples of the hybridizable DNA include least about 95% homology to the nucleotide sequence represented by SEQ ID NO: 3.

More specifically, as the DNA encoding the receptor protein containing the amino acid sequence represented by SEQ ID NO: 1, the DNA having the nucleotide sequence represented by SEQ ID NO: 3 or the like can be used.

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The DNA encoding the receptor protein having the same or substantially the same amino acid sequence represented by SEQ ID NO. 2 of the present invention may be, for example, DNA having the nucleolide sequence represented by SEQ ID NO: 4 or any DNA having a nucleotide sequence hybridizable to the nucleotide sequence represented by igand binding activity, signal information transmission activity and the like as those of the receptor protein peptide having the amino acid sequence represented by SEQ ID NO: 2. Examples of the hybridizable DNA include DNA having at least about 70% to about 80% homology, preferably, at least about 90% homology, more preferably, at least about SEQ ID NO: 4 under high stringent conditions and encoding a receptor protein which has the same activities, i.e., 95% homology to the nucleotide sequence represented by SEQ ID NO: 4,

More specifically, as the DNA encoding the receptor protein containing the amino acid sequence represented by SEQ ID NO: 2, the DNA having the nucleotide sequence represented by SEQ ID NO: 4 or the like can be used. The nucleolide sequence represented by SEQ ID NO: 4 is a variant of the nucleotide sequence of SEQ ID NO: 3 having an addition of 183 bases at its 5'-terminal.

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Hybridization can be carried out by a per se known method or its modification, for example, under high stringent

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60°C to about 65°C. In particular, hybridization conditions of sodium concentration at about 19 mM and a temperature The high stringent conditions used herein are, for example, those of sodium concentration at about 19 mM to about 40 mM, preferably about 19 mM to about 20 mM and a temperature at about 50°C to about 70°C, preferably about at about 65°C are most preferred.

The DNA encoding the partial peptide of the present invention may be any DNA in so far as it contains the nucleotide sequence encoding the above-described partial peptide of the present invention. The DNA may be any of genomic DNA, genomic DNA library, cDNA derived from the above-described cells and tissues, cDNA library derived from the above-described cells and tissues and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid, phagemid and the like. In addition, the DNA can be amplified by reverse transcriptase polymerase chain reaction (hereinalter abbreviated to RT-PCR) with a mRNA fraction prepared from the above-described cells and

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amino acid sequence represented by SEQ ID NO: 1 of the present invention may be, for example, DNA having the nucleotide sequence represented by SEQ ID NO: 3 or any DNA having a nucleotide sequence hybridizable to the nucleotide sequence represented by SEQ ID NO: 3 under high stringent conditions and encoding a receptor protein which has the same activities, i.e., ligand binding activity, signal information transmission activity and the like as those of the receptor protein peptide having the amino acid sequence represented by SEQ ID NO: 1. Examples of the hy-Specifically, the DNA encoding the partial peptide of the receptor protein having the same or substantially the same bridizable DNA include DNA having at least about 70% to about 80% homology, preferably, at least about 90% homology, more preferably, at least about 95% homology to the nucleotide sequence represented by SEQ ID NO: 3.

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be used. More specifically, as the DNA encoding the partial peptide having the amino acid sequences of the 78th to For example, as the DNA encoding the partial peptide of the receptor protein containing the amino acid sequence represented by SEQ ID NO: 1, the DNA having the nucleotide sequence represented by SEQ ID NO: 3 or the like can 130th amino acids, the 193rd to 204th amino acids, the 274th to the 307th amino acids or the 387th to 398th amino acids of the amino acid sequence represented by SEQ ID NO: 1, the DNA having the nucleotide sequence of the 232nd to 390th bases, the 577th to 612th bases, the 820th to 921st bases or the 1159th to 1194th bases of the nucleotide sequence represented by SEQ ID NO: 3 can be used. 8

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The DNA encoding the partial peptide of the receptor protein having the same or substantially the same amino sequence represented by SEQ ID NO: 4 under high stringent conditions and encoding a receptor protein which has acid sequence represented by SEQ ID NO: 2 of the present invention may be, for example, DNA having the nucleotide sequence represented by SEQ ID NO: 4 or any DNA having a nucleotide sequence hybridizable to the nucleotide the same activities, i.e., ligand binding activity, signal information transmission activity and the like as those of the receptor protein peptide having the amino acid sequence represented by SEQ ID NO: 2. Examples of the hybridizable DNA include DNA having at least about 70% to about 80% homology, preferably, at least about 90% homology, more preferably, at least about 95% homology to the nucleotide sequence represented by SEQ ID NO: 4.

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For example, as the DNA encoding the partial peptide of the receptor protein containing the amino acid sequence represented by SEQ ID NO: 2, the DNA having the nucleotide sequence represented by SEQ ID NO: 4 or the like can be used. More specifically, as the DNA encoding the partial peptide having the amino acid sequences of the 139th to 191th amino acids, the 254th to 265th amino acids, the 335th to the 368th amino acids or the 448th to 459th amino acids of the amino acid sequence represented by SEQID NO. 2, the DNA having the nucleotide sequence of the 415th to 573rd bases, the 760th to 795th bases, the 1003rd to 1104th bases or the 1342nd to 1377th bases of the nucleotide sequence represented by SEQ ID NO: 4 can be used.

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Hybridization can be carried out by a per se known method or its modification, for example, under high stringent conditions.

70°C, preferably about 60°C to about 65°C. In particular, hybridization conditions of sodium concentration at about 19 As described above, the high stringent conditions used herein are, for example, those of sodium concentration at about 19 mM to about 40 mM, preferably about 19 mM to about 20 mM, and a temperature at about 50°C to about mM and a temperature at about 65°C are most preferred.

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As the means for cloning the DNA encoding the entire receptor protein of the present invention, there is amplification by PCR using synthetic DNA primers containing partial nucleotide sequences of the receptor protein of the present Spring Harber Lab. Press, (1989). A commercially available library can be used according to the direction of the attached invention. Alternatively, DNA integrated into a suitable vector is selected by hybridization with labeled DNA fragment or a synthetic DNA encoding a part or entire region of the receptor protein of the present invention. The hybridization is carried out, for example, according to the method described in Molecular Cloning 2nd Ed., J. Samrook et al., Cold manufacurer's protocol.

Conversion of the nucleotide sequence of DNA can be carried out according to a per se known method such as Fakara Shuzo Co., Ltd., TM represents trademark). The nucleotide sequence represented by SEQ ID NO: 1 can be Gupped douplex method or Kunket method or its modification by using a known kit, Mutan™-G of Mutan™-K (both produced by deleting 183 based from the 5'-terminal of the nucleotide sequence represented by SEQ ID NO: 2.

The cloned DNA encoding the receptor protein can be used as such according to a particular purpose. Alternatively, if desired, it can be used after digestion with one or more restriction enzymes or a linker can be added. The DNA may have the codon, ATG, as a translation initiation codon at its 5' terminal side and the codon, TAA, TGA or TAG as a translation termination codon at its 3' terminal side. These translation initiation and termination codons can be added by using a suitable synthetic DNA adapter.

The expression vector of the receptor protein of the present invention can be prepared, for example, by (a) cutting out the desired DNA fragment from the DNA encoding the receptor protein of the present invention and (b) joining the DNA fragment to a suitable expression vector at the downstream from a promoter in the vector.

Examples of the vector include plasmids derived form <u>E. coli</u> (e.g., pBR325, pBR325, pUC12, pUC13), plasmids derived from <u>Becilius abblilis</u> (e.g., pUB110, pTP5, pC194), plasmids derived from yeast (e.g., pSH19, pSH15), bacteriophages such as Aphage, etc., animal viruses such as retrovirus, vaccinia virus, baculovirus, etc. as well as pA1-11, pRa/CMV, pRa/TRSV, pcDNAI/Neo, etc.

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The promoter used in the present invention may be any promoter in so far as it matches with a host to be used for gene expression. In case of using animal cells as the host, examples of the promoter include SRz promoter, SV40 cromoter, LTR promoter, CMV promoter, SV40 promoter, LTR promoter, CMV promoter, BV-TK promoter, etc. Among them, CMV promoter or SRz promoter include referred. In case of using bacteria of the genus <u>Escherichia</u> as the host, preferred examples of the promoter include rip promoter, lac promoter, resk promoter, AP, promoter, lpp promoter, etc. In case of using bacteria of the genus <u>Bactillus</u> as the host, preferred examples of the promoter, SPO2 promoter, penP promoter, etc. can be used. In case of using yeast as the host, preferred examples of the promoter include PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, etc. In case of using insect cells as the host, preferred examples of the promoter include polyhedrin prompter,

In addition to the above, optionally, the expression vector may further contains enhancer, splicing signal, poly A addition signal, selection marker, SV40 replication origin (hereinaller sometimes abbreviated to SV40 ori) etc. Examples of the selection marker include dihydrololate reductase (hereinaller sometimes abbreviated to dhirt) gene [methotrexate (MTX) resistance], ampicillin resistant gene (hereinaller sometimes abbreviated to Ampl), neomycin resistant gene (hereinaller sometimes abbreviated to Neo, G418 resistance), etc. In particular, when CHO (dhirr) cell is used together with DHFR gene as the selection marker, selection can also be carried out by using a thymidine free medium.

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If necessary, a signal sequence which matches with a host is added to the N-terminal side of the receptor protein of the present invention. As the signal sequence, there may be mentioned alkaline phosphatase signal sequence, OmpA signal sequence, etc. in case of using bacteria of the genus <u>Bectherichia</u> as the host; camplase signal sequence, subtilitish signal sequence, etc. in case of using bacteria of the genus <u>Bectility</u> as the host; making lactor or signal sequence, etc. in case of using bacteria of the genus <u>Bectility</u> as the host; making lactor or signal sequence, invertase signal sequence, etc. in case of using yeast as the host; insulin signal sequence, c-interferon signal sequence, antibody molecule signal sequence, atc. in case of using animal cells as the host, respectively.

The DNA encoding receptor protein of the present invention thus constructed can be employed to transform the host.

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As the host, for example, there may be mentioned bacteria of the genus Escherichia, bacterial of the genus Bactitus yeast, insect cells, insects and animal cells, etc.

Specific examples of bacteria of the genus <u>Escherichia</u> include <u>Escherichia coli</u> K12 DH1 [Proc. Natl. Acad. Sci. USA. <u>60</u>, 160 (1989)], JM103 [Nucleic Acids Research, <u>9</u>, 309 (1981)], JA221 [Journal of Molecular Biology, <u>170</u>, 517 (1978)], HB101 [Journal of Molecular Biology, <u>41</u>, 459 (1989)], C600 [Genetics, <u>39</u>, 440 (1954)], etc.

Examples of bacteria of the genus Bacilius include Bacilius subtilis MI114 (Gene, 24, 255 (1983)). 207-21 (Journal Bochemistry, 95, 87 (1984)), etc.

Examples of yeast include Saccaromyces cereviseae AH22, AH22; NA87-11A, DKD-5D, 20B-12, etc

- Examples of insect cells include <u>Spodoptera frugiperda</u> cell (SI cell), MG1 cell derived from mid-intestine of <u>Tri-</u>
 <u>choplusia ni</u>, High Five "v cell derived from egg of <u>Trichoplusia ni</u>, cells derived from <u>Mannestra brassicae</u>, cells derived from <u>Estigmena ecroe</u>, etc. for the virus, AcNPV; and <u>Bombyx mori</u> N cell (BmN cell), etc. for the virus, BmNPV. As the SI cell, for example, SI9 cell (ATCC CRL1711) and SI21 cell described by Vaughn, J. L., in Vitro, <u>13</u>, 213-217 (1977) can be used.
- As the insect, for example, a larva of <u>Bombyx mori</u> can be used [Maeda et al., Nature, <u>315</u>, 592 (1985)].

 Examples of animal cells include monkey cell COS-7, Vero, Chinese hamster cell CHO, DHFR gene deficient Chinese hamster cell CHO (dhir CHO cell), mouse L cell, mouse AlT-20, mouse myeloma cell, rat GH 3, human FL

Transformation of becteria of the genus <u>Escherichia</u> is carried out, for example, according to the method described in Proc. Natl. Acad. Sci. USA, <u>59</u>, 2110 (1972) or Gene, <u>17</u>, 107 (1982).

55 Transformation of bacteria of the genus <u>Bacillus</u> is carried out, for example, according to the method described in Molecular & General Genetics, <u>158</u>, 111 (1979).

Transformation of yeast is carried out, for example, the method described in Proc. Natl. Acad. Sci. USA, 75, 1929 (1978).

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Transformation of insect cells or insect is carried out, for example, according to the method described in Biol Technology, 6, 47-55(1988).

Transformation of animal cells is carried out, for example, according to the method described in Virology, $\underline{52}$, 456 (1973).

Thus, the transforment transformed with the expression vector containing the DNA encoding the G-protein coupled receptor protein can be obtained.

in case of the bacterial host of the genus <u>Escherchia</u> or <u>Bacillus</u>, the transformant can be suitably cultivated in a liquid cultive medium and materials required for growth of the transformant such as carbon sources, nitrogen sources, increases a carbon sources, nitrogen sources, increases a carbon sources, nitrogen sources, increases a carbon sources, nitrogen sources, and the carbon sources are carbon sources.

inorganic materials, etc. are added to the medium. Examples of the carbon sources include glucose, dextrin, soluble starch, sucrose, etc. The nitrogen sources include, for example, fronganic materials such as ammonium salts, nitrate salts, com steep liquor, peptone, casein, meat extract, soybean meat, potato extract, etc. The inorganic materials include, for example, calcium chloride, sodium dihydrogen phosphate, megnesium chloride, etc. In addition, yeast, vitamins, growth promoting factors etc. can be added. Preferably, the medium is adjusted to pH about 5 to about 6.

Preferably, the medium for cultivating the bacteria of the genus <u>Escherichia</u> is, for example, M9 medium containing glucose and Casamino Acids (Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York, 1972). If necessary, in order to activate the promoter efficiently, for example, an agent such as 3p-indolyl acrylic acid can be added to the medium.

in case of the bacterial host of the genus <u>Escherichia</u>, normally, the transformant is cultivated at about 15°C to about 43°C for about 3 hours to about 24 hours. If necessary, the culture can be aerated or stirred.

In case of the bacterial host of the genus <u>Bacillus,</u> normally, the transformant is cultivated at about 30°C to about 40°C for about 6 hours to about 24 hours. If necessary, the culture can be aerated or stirred.

In case of the yeast host, the transformat is cultivated in, for example, Burkholder's minimal medium [Bostian, K. L. et al., Proc. Natl. Acad. Sci. USA, <u>77</u>, 4505 (1980) and SD medium containing 0.5% Casamino Acids [Bitter, G. A., Proc. Natl. Acad. Sci. USA, <u>81</u>, 5330 (1984)]. Preferably, the medium is adjusted to pH about 5 to about 8. Normally, the transformant is cultivated at about 20°C to about 35°C for about 24 hours to about 72 hours. If necessary, the

culture can be aerated or stirred.

In case of the insect cell host or insect host, the transformat is cultivated in, for example, Gace's insect Medium (Grace, T. C. C., Nature, <u>195</u>, 788 (1982)] to which an appropriate additive such as inactivated 10% bovine serum is added. Preferably, the medium is adjusted to pH about 6.2 to about 6.4. Normally, the transformant is cultivated at about 27°C for about 3 days to about 5 days and, if necessary, the culture can be serated or atfreed.

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In case of the animal cell host, the transformant is cultivated in, for example, MEM medium containing about 5% to about 20% fetal bovine sorum [Science, 122, 501 (1852)], DMEM nedium [Virology, §, 396 81959)], RPMI 1640 medium [The Journal of the American Medical Association, 199, 519 (1987)], 199 medium [Proceeding of the Society for the Biological Medicine, 73, 1 (1950)], etc. Preferably, the medium is adjusted to pH about 6 to about 8. Normally, the transformant is cultivated at about 30°C to about 40°C for about 15 hours to about 60 hours and, if necessary, the culture can be aerated or stirred.

As described hereinabove, the G-protein coupled receptor protein of the present invention can be produced at the cell membrane of the transformant.

Separation and purification of the receptor protein of the present invention from the above culture can be carried out, for example, as follows.

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Extraction of the receptor protein of the present invention from the transformant culture can be carried out by an appropriate known method. For example, after cultivation, the transformant is recovered by a per se known method and suspended in a suitable buffer. Then, the transformant is disrupted by a per se known method such as ultrasonication, treatment with lysozyme and/or freeze-thaw cycling, followed by separating a crude extract of the receptor protein by centiflugation, filtration, etc. The buffer may contain a protein modifier such as urea, guanine hydrochloride or a surfactant such as Triton X-100rd, etc. When the receptor protein is secreted in the culture both, after completion of cultivation, its supermatant can be separated from the transformant cells to collect the supermatant.

Purilication of the receptor protein contained in the culture supernatant thus obtained or the extract can be carried out by combining per se known separation and purification methods appropriately. As the per se known separation so and purification methods, there may be mentioned a method utilizing difference in solubilities such as salting out, solvent precipitation, etc.; a method mainly utilizing difference in molecular weights such as dataysis, utrafiltration, get selected to the second of the se

etc; a method utilizing difference in iscelectric points such as iscelectric point electrophoresis; and the like. When the free receptor protein is obtained, it can be converted into its sall by a per se known method or its modification. On the other head, when the receptor protein is obtained in the form of a salt, it can be converted into the free receptor protein or a different salt by a per se known method or its modification.

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The receptor protein produced by the recombinant can be treated with an appropriate protein modifying enzyme prior to or after purification to appropriately modify the protein or to partially remove a polypeptide. Examples of the protein modifying enzyme include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase and the

The activity of thus-produced receptor protein or its sall of the present invention can be determined by a binding test with a labeled ligand or an enzyme immunoassay with a specific antibody.

The receptor protein, its partial peptide or their salts, and DNAs encoding them of the present invention can be employed (a) in a method for determination of a figand to the receptor protein of the present invention, (b) for construction of a expression system of the recombinant receptor protein, (d) for development of an autiserum, (c) for construction of a expression system of the recombinant receptor protein, (d) for everyphenent of an receptor binding assay system and excreening for candidate compounds for drugs using the expression system, (e) for practice of drug design based on comparison with structurally analogous ligands and receptors, (l) as reagons for preparation of probes to be used in gene diagnosis, PCR primers, etc., (g) as drugs for gene prophylaxis and therapy, and the like.

In particular, screening for agonists or antagonists to the G-protein coupled receptor protein which are specific to a human being and another mammal can be carried out by using a receptor binding assay system utilizing an expression system of the recombinant G-protein coupled receptor protein of the present invention, and the agonists and antagonists can be used as prophylactic and therapeutic drugs for various diseases.

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More specific description of the use of the receptor protein, its partial peptide or their safts, DNAs encoding the receptor protein or its partial peptide and antibody will be set forth below.

(I) Determination method of a ligand to the G-protein receptor protein

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The receptor protein of the present invention or its salt, or the partial protein or its salt of the present invention is useful as a reagent for investigation or determination of a ligand to the receptor protein or its salt of the present invention provides a method for defermination of a ligand to the receptor protein of the present invention provides an emblod for defermination of a ligand to the receptor protein of the present invention or a salt intend into

That is, the present invention provides a method for determination of a figand to the receptor protein of the present invention comprising bringing the receptor protein or its partial peptide of the present invention or a salt thereof into contact with a test compound.

Examples of the test compound include itssue extract, cell culture supermatant of a human being or another mam-

Examples of the test compound include listue avitact, can cluture supermetent or a futuran tentral undernited many or arbitral many many for a mouse, rat, pig, cattle, sheep, monkey, atc.), or he like, in addition to the above-described known ligands, such as angoleons, bornbesin, cannablord, cholecystokinin, gittamine, serotomin, melatomin, neuropeptide Known ligands, purine, vasopressin, oxylocch, PACAP, secretin, gitcagon, calcitonin, adrenomedullin, somatostatin, GHPH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal and related polypeptides), doparnine, motilin, arrylin, bradykinin, CGRP (calcitonin gene related proteins), leukoritene, pencreasitacin, prostagiandin, thromboxane, adenosisie, adenablin, or of schorinomy less, and serotomy services and services are also services and services and services and services are adenositie, and gallamine. For example, the tissue extract or the cell culture supormatant is added to the receptor protein of the present invention and the mixture is fractionated by measuring a cell stimulation activity, etc. to finally obtain a single ligand.

Specifically, the ligand determination method of the present invention is carried out by using the receptor protein or its partial peptide of the present invention or a salt thereof, or by constructing an expression system of the present invention or a salt thereof, or by constructing an expression system of the recombinant receptor protein and using a receptor binding assay system utilizing the expression system to determine a compound (e.g., peptide, protein, non-paptide compound, synthetic compound, emeration product, etc.) showing a cell stimu-lation activity to enhance or inhibit release of arachidonic acid, release of acely choline, release of intracellular CAMP, formation of intracellular cAMP, formation of intracellular cAMP, the receiptor of investion by the present invention.

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The ligand determination method of the present invention is characterized by measurament of, for example, an amount of a test compound bound to the eceptor protein or the partial peptide or a cell stimulation activity upon bringing the receiptor protein or the partial peptide into contact with the test compound.

More specifically, the ligand determination method of the present invention is:

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(a) a method for delemination of a ligand to the receptor protein or its sall of the present invention which comprises labeling a test compound, and measuring an amount of the labeled test compound bound to the receptor protein, the partial peptide or their salts upon bringing the receptor protein, the partial peptide or their salts into contact with the labeled test compound;

(b) a method for determination of a figand to the receptor protein or its sall of the present invention which comprises labeling at lest compound, and measuring an amount of the labeled test compound bound to cells containing the receptor protein or a membrane fraction of the cells upon bringing the labeled test compound into contact with the cell or the cell membrane fraction.

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(c) a method for determination of a figand to the receptor protein which comprises labelling a test compound, and measuring an amount of the labeled test compound bound to the G-protein coupled receptor protein expressed on the cell membrane of a transformant containing the DNA encoding the receptor protein of the present invention by cultivating the transformant upon bringing the labeled test compound into contact with the expressed G-protein

coupled receptor protein;

(d) a method for determination of a ligand to the receptor protein or its salt of the present invention which comprises (d) a method for determination activity (e.g., an activity to enhance or inhibit release of arachidonic acid, release of measuring a cell stimulation activity (e.g., an activity to enhance or inhibit release of varachilations activity intracellular CAMP, formation of intracellular cAMP, formation of intracellular cAMP, production of intracellular change of cell membrane potential, phospholylation of intracellular protein, activation of c-tes, lowering of pt, etc.) mediated by the receptor protein upon bringing a test compound into contact with cells containing the receptor protein.

(e) a mathod for determination of a ligand to the receptor protein or its satt which comprises which comprises measuring a cell stimulation activity (e.g., an activity to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of intracellular CEAPs, formation of infracellular cAMPs, formation of insition prosphate, change of cell immembrane operating, thospholytation of intracellular protein, activity of colors, lowering of ptt, etc.) mediated by the receptor protein appropriate in the celeptor protein expressed on the cell membrane of a transformant containing DNA encoding the receptor protein of the present invention by cultivating the transformant.

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20 The receptor protein used in the ligand determination of the present invention may be any protein in so far as it contains the above-described receptor protein or partial peptide of the present invention. In particular, the receptor protein expressed in a large amount by using animal cells is suitable.

For producing the receptor protein of the present invention, the above-described expression process can be used. In particular, it is preferred to carry out expression of the DNA encoding the receptor protein in cells of mammals of insects. Normally, cDNA is used as a DNA fragment encoding the desired part of the protein, but the present invention is not necessarily infined to this. For example, a gene fragment or a synthetic DNA can also be used. For transferring a DNA tragment encoding the receptor protein of the present invention find a host animal cell and expressing it efficiently. It is preferred to integrate the DNA fragment into the downstream from a polyhetin promoter of nuclear polyhetosis virus (NPV) belonging to baculovirus whose host is insects, a promoter darived from SV40, retrovirus promoter metallothionein promoter, human heat shock promoter, cytomogatovirus promoter, and qualitative assay of expression of the receptor protein can be carried out by a per se known method. For example, the assay can be carried out according to the method described by Nambi, P. et al., J. Blot, Chem., 267, 19555-1958 (1992).

In the ligand determination of the present invention, the receptor protein, its partial peptide or their salts may be
the receptor protein, its partial peptide or their salts as such which are purified according to a per se known method,
or a material containing the receptor protein, its partial peptide or their salts such as cells containing the receptor protein
or their membrane fractions.

In case of using the cells containing the receptor protein of the present invention, they may be immobilized with glutaraldehyde, formalin, etc. immobilization can be carried out by a per se known method.

guaraneanyor, runnani, ste. Information of the present invention are host cells expressing the receptor protein of The cells containing the receptor protein of the present invention are host cells and invention. The Examples of the host cells include E. coli. Bacillus subtilis, yeast, insect cells, animal cells and the like.

The cell membrane fraction is a fraction containing many cell membranes obtained by disrupting cells and then treated by a per se known method. The disruption of cells can be carried out, for example, using Potter-Elvehijem homogenizer, Waring blender or Polytron (Kinematica), ultrasonication, French press, etc. Fractionation of the cell membrane iraction can mainly be carried out by fractionation centrifugation, density-gradient centrifugation of the like. For example, a disrupted cell suspension is centrifugat at a bow rate (500 pm-3000 pm) for a short period of time (normally, about 1 minute-10 minutes) and further the supernatant is centrifugat at a high rate (15000 pm-30000 pm) for about 30 minutes to about 2 hours to obtain a cell membrane fraction as precipitate. This cell membrane fraction contains the expressed receptor protein and many membrane components such as phospholipids, membrane proteins

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moverund of the receptor protein contained in the cells or the cell membrane traction is preferably 10° to 10° molecules, more preferably 10° to 10° molecules, more preferably 10° to 10° molecules per one cell. As the expression level is higher, a ligand binding activity (specific activity) becomes higher, which makes not only construction of a high sensitive screening system but also determination of a large number of samples in one lot possible.

vocamination or many approximation methods (a) to (c), a suitable receptor protein fraction and a labeled test compound are required.

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The receptor protein fraction is preferably a naturally occurring receptor protein fraction or a recombinant receptor

used herein means the equivalent ligand binding activity, signal information transmission activity or the like. prolein fraction having the equivalent activity to that of the naturally occurring receptor prolein. The equivalent activity

NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.), endothelin, enternoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, ogastrin, histamine, neurotensin, TRH, pancreatic polypeptides or gallamine labeled with [4H], [126], [4C], [55], etc. pancreastacin, prostaglandin, thromboxane, adenosine, adrenalin, α or β -chemokine (e.g., IL-8, GRO α , GRO β , GRO γ , and related polypeptides), dopamine, motifin, armylin, bradykinin, CGRP (calcitonin gene related proteins), leukotriene, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal The labeled test compound is preferably the above-exemplified ligand such as angiotensin, bombesin, cannabi-

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25 beled test compound is added, is provided. The reaction is carried out at about 0°C to about 50°C, preferably about or the like. In order to minimize non-specific binding, a surfactant such as CHAPS, Tween-80™ (Kao-Atlas), digitonin the receptor protein can be used, for example, a phosphate buffer of pH 4 to 10, preferably pH 6 to 8, Tris-HCl buffer method to prepare a receptor standard. Any buffer which does not interfere with the binding between the ligand and fraction containing the receptor protein of the present invention are suspended in a buffer satiable for the determination ng degradation of the receptor protein an higand by a protease, a protease inhibitor such as PMSF, leupsplin, E-64 (Peptide Kenkyu-sho), pepstalin, etc. can also be added to the buffer. A given amount (5000 cpm-500000 cpm) of the non-specific binding (NSB) from the total binding amount (B) exceeds 0 cpm can be selected as the ligand to the For evaluating an amount of non-specific binding (NSB), a reaction tube, to which a large excess amount of the unlatest compound labeled with [3 H], [4 C], [4 C], [3 S] or the like is added to 0.01 ml to 10 ml of the receptor suspension. suitable amount of the same buffer and the radioactivity remaining in the glass fiber filter paper is counted with a liquid scrintilization counter or a y-counter. The test compound whose count (B-NSB) obtained by subtracting the amount of pletion of the reaction, the reaction mixture was filtered through, for example, glass fiber filter paper, washed with a 4°C to about 37°C for about 20 minutes to about 24 hours, preferably about 30 minutes to about 3 hours. After comsoxycholate, etc. and various proleins such as bovine serum albumin, gelatin, etc. to the buffer. In addition, tor inhib Specifically, for carrying out the ligand determination method of the present invention, the cells or cell membrane

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of an index compound of a cell stimulating activity (e.g., arachidonic acid, etc.) is difficult due to a degradation enzyme brane potential, phospholylation of intracellular protein, activation of c-los, lowering of pH, etc.) mediated by the receptor g., activity to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of intracellular Ca2. as for cAMP production inhibitory activity or the like, the activity can be detected as the production inhibitory activity contained in the cells, the assay can be carried out with addition of an inhibitor of the degradation enzyme. In addition, determine a product formed by a method suitable for determination of the product. Where an assay of the formation the culture is incubated for a given period of time, followed by extracting the cells or recovering the supernatant to is replaced with a fresh medium or a suitable buffer which does not have cytoloxicity. After addition of a test compound, the cells containing the receptor protein are cultivated in a well plate, etc. For ligand determination, the culture medium protein of the present invention by a known method or a commercially available determination kit. Specifically, first, formation of intracellular cAMP, formation of intracellular cGMP, production of inositol phosphate, change of cell memreceptor protein or its salt of the present invention. The above ligand determination methods (d) and (e) can be carried out by measuring a cell stimulation activity (e

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of cells whose basic production is increased with forskolin, etc. coplor prolein of the present invention or a salt thereof, a cell membrane fraction of cells containing the receptor an essential component the receptor protein or the partial peptide of the present invention, cells containing the The determination kit of a ligand which binds to the receptor protein or its salt of the present invention comprises

bratein of the present invention, or the like. Examples of the kid for ligand determination of the present invention include as follows

(1) Reagent for ligand determination

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(a) Measurement buffer and washing buffer

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is can be prepare when it is used (Gibco). The buffer is sterilized by filtration through a filter of 0.45 µm in pore diameter and then stored at 4°C or A buller obtained by addition of 0.05% of bovine serum albumin (Sigma) to Hanks' balanced selt solution

(b) Standard of G-protein coupled receptor protein

5 x 10⁵ cells/well in a 12-well plate and cultivated at 37°C for 2 days in 5% CO_{2*}95% air to obtain a standard of the receptor protein. CHO cells expressing the receptor protein of the present invention are subjected to passage in an amount of

(c) Labeled test compound

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An aqueous solution of the labeled test compound is stored at 4°C or -20°C and, when it is used, it is diluted A test compound labeled with a commercial available [AH], [125], [14C], [35S], etc. or another suitable label

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to 1 µM with the measurement buffer. As for a water-insoluble or slightly water-soluble compound, the compound is dissolved in dimethyltormamide, DMSO, methanol, etc.

(d) Non-labeled test compound

times concentration. The same test compound as that of the labeled test compound is used to prepare a solution in 100 to 1000

(2) Measurement

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ml of the measurement buffer and then 490 μl of the buffer is added to the respective wells. (a) The receptor protein expressing CHO cells cultivated in a 12-well tissue culture plate is washed twice with 1

labeled test compound bound to the cells is dissolved with 0.2 N NeOH-1% SDS and mixed with 4 mt of liquid (c) The reaction mixture is removed and the wells are washed three times with 1 ml of the washing buffer. The (b) 5 µl of the labeled test compound is added and reacted at room temperature for one hour. scintillator A (Wako Pure Chemical Industries, Ltd.).

35 (d) Radioactivity is measured by a liquid scintillation counter (Beckman)

8 oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP in brain, pituitary, pancreas, etc. Specific examples thereof include the above-exemplified ligands, that is, angiotensin, (vasoactive intestinal and related polypeptides), dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptides or gallamine. 8, GRO¢, GROβ, GROγ, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1¢, MIP-1β, FANTES, proteins), laukatriene, pancreastacin, prostagiandin, thromboxane, adenosine, adrenatin, lpha or eta-chemokine (e.g., IL-The ligand which can bind to the receptor protein of the present invention is, for example, that specifically present

(II) Gene prophylactic and therapeutic drug of the G-protein coupled receptor protein deliciency diseases

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છ by deficiency of the G-protein coupled receptor protein deficiency depending upon a particular activity of the ligand. receptor protein of the present invention can be used as a drug for gene prophylaxis and therapy of diseases caused If a ligand to the receptor protein of the present invention is found out by the above method (I), DNA encoding the

ક્ષ decrease in the receptor protein of the present invention, the amount of the receptor protein in the patient's body can present invention into subject cells to express it, followed by transplantation of the cells into the patient. Then, DNA be increased to exhibit the sufficient activity of the ligand, for example, by (i) administering DNA encoding the receptor encoding the receptor protein of the present invention is useful as a safe and low toxic drug for gene prophylaxis and protein of the present invention to the patient to express it, or (ii) inserting DNA encoding the receptor protein of the For example, when there is a patient whose physiological activity of a ligand is scarcely expected because of

therapy of disease caused by deficiency of the receptor prolein of the present invention. DNA encoding the receptor protein of the present invention (hereinafter sometimed abbreviated to the DNA of the

present invention) alone or, after inserted into a suitable vector such as retroviral vector, adenoviral vector, adenovirus for example, one or more pharmaceutically acceptable carriers, flavors, excipients, vehicles, preservatives, stabilizers coaling, capsules, elixirs, microcapsules, etc. for oral administration, or in the form of injectable preparations such as associated viral vector, etc., can be used as the above prophylactic and therapeutic drug according to a conventional method. For example, the DNA of the present invention can be used in the form of tablets, if necessary, providing sugar aseptic solutions or suspensions in water or other pharmaceutically acceptable solutions for parenteral administration. A pharmaceutical composition in a unit dosage form can be prepared by mixing the DNA of the present invention with,

such an amount that a dose in the intended desired range can be obtained. binders, etc. according to generally acceptable manner. The effective component is contained in the composition in

g g peppermint, akamono oil and cherry, and the like. In case of the capsule dosage unit form, in addition to the above acid, lubricants such as magnesium stearate, sweetenings such as sucrose, lactose and saccharin, flavors such as gum and gum arabic, excipients such as crystalline cellulose, swelling agents such as com starch, gelatin and alginic sodium chloride, etc.) and sullable dissolution aids, for example, alcohols (e.g., ethanol), polyalcohols (e.g., propylene include physiological saline, isotonic solutions containing glucose and other adjuvants (e.g., D-sorbitol, D-mannitol water and a natural vegetable oil such as sesame oil, coconut oil, etc. Examples of the injectable aqueous solution to a conventional manner, for example, by dissolving or suspending the active component in a vehicle such as injectable component, it can contain a liquid carrier such as fat or oil. An injectable aseptic composition can be prepared according Examples of additives to be mixed in tablets, caupsels, etc. include binders such as gelatin, corn starch, tragacanth

benzyl alcohol, etc. can be further added.

The above prophylactic and therapeutic drugs can further contain, for example, buffers (e.g., phosphate buffer, socialme agelated agents (e.g., benzalkonium chloride, proceine hydrochloride, etc.), stabilizers (e.g., burna beating agents) (e.g., benzalkonium chloride, proceine hydrochloride, etc.), stabilizers (e.g., burna beating buffer, burna beating buffer, and proceined in the like. The injectable preparation thus proclused is normally filled in a suitable empoule. Since the pheramecultical composition thus obtained is safe and low toxic, it can be administrate a suitable empoule. Since the pheramecultical administrated is safe and low toxic, it can be administrated and another mammal (e.g., rat, abbit), it seate, oils, cartile, cat, dog, monkey, etc.). Although the amount of the DNA of the present invention to be in general, for oral administration to an adult human being (as 60 kg body weight), the sidning administration is administration in an adult human being (as 60 kg body weight), it is administration in a bout 100 mg/day, preferably about 10 mg/day to about 30 mg/day, preferably about 0 an adult human being (as 60 kg body weight), it is day to about 30 mg/day, preferably about 0.1 mg/day to about 30 mg/day, preferably about 0.1 mg/day to about 30 mg/day, preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about 0.1 mg/day to about 30 mg/day, more preferably about

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(III) Gene diagnosing agent

The DNA encoding the receptor protein or the partial peptide of the present invention can be used for detecting an abnormality of DNA encoding the receptor protein or the partial peptide of the present invention (abnormal gene) in a human being or another mammal (e.g., rat, rabbit, sheep, pig, cattle, cat, dog, monkey, etc.). Therefore, the DNA encoding the receptor protein or the partial peptide of the present invention is useful as a gene diagnosing agent for detecting abnormality of the DNA.

(IV) Determination method of ligand to the G-protein coupled receptor protein

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The receptor protein, the partial peptide and their salts of the present invention have ligand binding properties and they can be used for determination of ligand concentration in the living body in a high sensitivity.

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The method for determination of the present invention can be used, for example, in combination with a competitive method. That is, the ligand concentration can be determined by bringing a specimen into contact with the receptor protein, the partial poptide or a salt thereof of the present invention. Specifically, the determination method can be admined out, for example, according to the methods described in Hinselt inte. Ed., "Radioirmunoassay", Kodan-sha, 1979 or finel modifications.

(V) Method for screening for compounds which after binding of a ligand to the G-protein coupled receptor protein

Compounds which alter binding of a ligand to the receptor protein or its saft (e.g., peptides, proteins, non-paptide compounds, synthetic compounds, lementation products) can be screened efficiently by using the receptor protein the partial peptide or their state of the present invention, or by constructing an expression system of the recombinant receptor protein and using a receptor binding assay system utilizing the expression system. Examples of these compounds include compounds having cell situation activities (e.g., activity to enhance or inhibit release or arechiconic case) production of innsitiol phosphale, change of cell membrane potential, phospholytation of intracellular case) production of innsitiol phosphale, thet.) mediated by the receptor protein of the present invention) and compounds which do not have such activities (i.e., so-called agonists anlagonists to the receptor protein of the present invention) and compounds which do not have such activities (i.e., so-called agonists anlagonists to the receptor protein of the present invention).

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That is, the present invention provide a method for screening for compounds which after binding of a ligand to the receptor protein or its eatt of the present invention, or their salts which comprises comparing (i) binding of the ligand to the receptor protein or the partial peptide of the present invention or a salt thereof upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof with the figand, and (ii) that upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into contact with the figand, and (ii) that upon bringing test compound.

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In the screening method of the present invention, an amount of the ligand bound to the receptor protein, the partial peptide or a salt thereof, a cell stimulation activity or the like is measured and compared upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into conlact with the ligand, and (ii) that upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into contact with the ligand and a lest commonner.

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More specifically, the screening method of the present invention includes:

(a) a method for screening for compounds which after binding of a ligand to the receptor protein or its salt of the present invention, or their salts which comprises labeling the ligand, and measuring and comparing (f) an amount of the labeled ligand bound to the receptor protein or the partial peptide of the present invention or a salt thereof upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into contact with the labeled ligand, and (ii) that upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into contact with the labeled ligand and a test compound;

(b) a method for screening for compounds which alter binding of a figand to the receptor protein or its salt of the present invention, or their eastle which comprises labeling the ligand, and measuring and comparing (f) an amount of the labeled figand bound to cells containing the receptor protein or an embrane fraction of the cells upon bringing the labeled figand into contact with the cells or the membrane fraction, and (ii) that upon bringing the labeled ligand and a test compound into contact with the cells or the membrane fraction, and (ii) that upon bringing the labeled ligand and a test compound into contact with the cells or the membrane fraction,

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(c) a mothod for screening for compounds which after binding of a ligand to the receptor protein or its salt of the present invention, or their salts which comprises labeling the ligand, and measuring and comparing (f) an amount of the tabeled tigand bound to the receptor protein expressed on the cell membrane of a transformant containing DNA encoding the receptor protein of the present invention by cutivating the transformant upon bringing the labeled ligand into contact with the expressed receptor protein, and (ii) that upon bringing the labeled ligand and a test contact with the expressed receptor protein;

(d) a method for screening for compounds which after binding of a ligand to the receptor protein or its salt of the present invention, or their salts which comprises measuring and companing (i) a cell stimulation activity (e.g., activity to enhance or inhibit rolease of arechidonic acid, rolease of accept choins, rolease of intracellular Ca2*, formation of intracellular choins, intracellular choins are compounded in the receptor potential, phosphylation of intracellular protein, activation of crios, lowering of pt, etc.) mediated by the rocaptor upon bringing a compound which activates the receptor protein of the present invention (e.g., a ligand to the receptor protein of the present invention (e.g., a ligand to the receptor protein of the present invention into contact with cells containing the receptor protein and a test compound into contact with the cells.

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(e) a method for screening for compounds which alter binding of a ligand to the receptor protein or its salt of the present invention, or their salts which comprises measuring and comparing (i) a cell stimulation activity (e.g., activity to enhance or inhight release of arachidonic acid, rebease of arealy choline, release of intacellular Ca2*-, formation of intacellular cAAP, formation of intracellular cAAP, production of incastol phosphate, change of cell membrane potential, phospholylation of intracellular protein, activation of c-dcs, lowering of pht, etc.) madated by the receptor upon bringing a compound which activates the receptor protein of the present invention (e.g., a ligand a transformant confaring DNA encoding the receptor protein by cultivaling the transformant, and (ii) that upon bringing the compound with a streams are receptor protein by present invention and a test compound with activates the receptor protein by present invention and a test compound into contact with the receptor protein or the present invention and a test compound into

Before the receptor protein of the present invention is available, for screening for a G-protein coupled receptor agonist or aniagonist, first, it is necessary to obtain candidate compounds by using cells, lissues or a cell membrane inaction thereof containing the G-protein coupled receptor protein of rat, etc (primary screening) and then to confirm whether or not the candidate compounds actually inhibit the binding of human G-protein coupled receptor protein and alignad (secondary screening). When cells issues or a cell membrane thereof are used as such, other receptor proteins are present, which make screening). When cells insues or a cell membrane thereof are used as such, other receptor proteins are present, which make screening for an aponist or aniagonist to the objective receptor protein difficult. However, for example, if the human receptor protein of the present invention is used, the primary screening is not required and efficient screening for compounds which inhibit the binding of a ligand to the G-protein coupled receptor protein can be carried out. In addition, whether the compound thus screened for is an agonist or an aniagonist can be readily evaluated.

The receptor protein used in the screening method of the present invention may be any protein in so far as it contains the above-described receptor protein or partial peptide of the present invention. A cell membrane fraction of a mammalian internal organ containing the receptor protein of the present invention is suitable. However, in particular, the fitternal organs of a human being is hardly available and therefore the receptor protein is suitable. However, in particular, by using a recombinant is suitable for the screening.

For producing the receptor protein of the present invention, the above-described expression process can be used. In particular, it is preferred to carry out expression of the DNA encoding the receptor protein in cells of mammals or insects. Normally, cDNA is used as a DNA fragment encoding the desired part of the protein, but the present invention is not necessarily limited to this. For example, a gene fragment or a synthetic DNA can also be used. For transferring

it is preferred to integrate the DNA tragment into the downstream from a polyhetrin promoter of nuclear polyhetosis talive and qualitative assay of the expressed receptor can be carried out by a per se known method. For example, the virus (NPV) belonging to baculovirus whose host is insects, a promoter derived from SV40, retrovirus promoter, meta DNA tragment encoding the receptor protein of the present invention into a host animal cell and expressing it efficiently, assay can be carried out according to the method described by Nambi, P. et al., J. Biol. Chem., 267, 19555-19559 allothionein promoter, human heat shock promoter, cytomegalovirus promoter, SRα promoter or the like. The quanti-

material containing the receptor protein, its partial peptide or their selts such as cells containing the receptor protein receptor protain, its partial peptide or the salt as such which is purified according to a per se known method, or a In the screening method of the present invention, the receptor protein, its partial peptide or their salts may be the

or their membrane fractions In case of using the cells containing the receptor protein of the present invention, they may be immobilized with

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glularaldehyde, formalin, etc. Immobilization can be carried out by a per se known method. te present invention. Preferrad examples of the host cells include E. coli, Bacillus subtills, yeast, insect cells, anima The cells containing the receptor protein of the present invention are host cells expressing the receptor protein of

ation of the cell membrane fraction can mainly be carried out by fractionation centrifugation, density-gradient centriftreated by a per se known method. The disruption of cells can be carried out, for example, using Potter-Eivehjem homogenizer, Waring blender or Polytron (manufactured by Kinematica), ultrasonication, French press, etc. Fractionfraction contains the expressed receptor protein and many membrane components such as phospholipids, membrane ugation or the like. For example, a disrupted cell suspension is centriluged at a low rate (500 rpm-3000 rpm) for a short 30000 rpm) for about 30 minutes to about 2 hours to obtain a cell mambrane fraction as a pracipitate. This cell membrane period of time (normally, about 1-10 minutes) and further the supernatant is centrifuged at a high rate (15000 rpmalls and the like. The cell membrane fraction is a fraction containing many cell membranes obtained by disrupting cells and then

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is higher, a ligand binding activity (specific activity) becomes higher, which makes not only construction of a high senfraction is preferably 10³ to 10⁸ molecules, more preferably 10⁵ to 10⁷ molecules per one cell. As the expression level proteins and the like. The amount of the receptor protein contained in the cells containing the receptor protein or the cell membrane

မွ compound are required. The receptor protein fraction is preferably a naturally occurring receptor protein fraction or a The equivalent activity used herain means equivalent ligand binding activity, signal information transmission activity recombinant receptor protein fraction having the equivalent activity to that of the naturally occurring receptor protein silve screening system but also determination of a large number of samples in one lot possible. For carrying out the above screening methods (a) to (c), a suitable receptor protein fraction and a labeled test

છ The labeled ligand includes labeled ligands and labeled ligand enalog compounds. For example, the ligand labeled

a receptor protein standard. Any butter which does not interfere with the binding between the ligand and the receptor containing the receptor protein of the present invention are suspended in a buffer satisble for the screening to prepare with [3H], [125]], [14C], [35S], etc. can be used. protein can be used, for example, a phosphate buffer of pH 4 to 10, preferably pH 6 to 8, Tris-HCl buffer or the like. In by Peptide Kenkyu-sho), pepsialin, etc. can also be added to the buffer. A given amount of the labeled ligand (5000 🖏 the receptor protein and the ligand by a protease, a protease inhibitor such as PMSF, laupeptin, E-64 (manutactured a glass fiber filter paper, washed with a suitable amount of the same buffer and the radioactivity remaining in the glass about 0°C to about 50°C, preferably about 4°C to about 37°C for about 20 minutes to about 24 hours, preferably about reaction tube to which a large excess amount of the unlabeled ligand is added is provided. The reaction is carried out a test compound is present in the receptor suspension. For evaluating an amount of non-specific binding (NSB), a cpm-500000 cpm) is added to 0.01 ml to 10 ml of the receptor suspension and, at the same time, 10⁴ M to 10⁻¹⁰ M of 30 minutes to about 3 hours. After completion of the reaction, the reaction mixture was filtered through, for example yder to minimize non-specific binding, a surfactant such as CHAPS, Tween-80™ (Kao-Atlas), digitonin, deoxycholate, is, and various proteins such as bovine serum albumin, gelatin, etc. to the buffer, in addition, for inhibiting degradation Specifically, for carrying out the screening method of the present invention, the cells or cell membrane fraction

to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of infracellular Ce2+, formation of phospholylation of intracellular protein, activation of c-tos, lowering of pH, etc.) mediated by the receptor protein of the intracellular cAMP, formation of intracellular cGMP, production of inositol phosphate, change of cell membrane potential without any antagonistic material as 100%. The above screening methods (d) and (e) can be carried out by measuring a cell stimulation activity (e.g., activity

taking the count (B₀-NSB) obtained by subtracting the count of non-specific binding (NSB) from the count (B₀) obtained count (B-NSB) is 50% or lower can be selected as a candidate compound capable of inhibiting antagonism, when

fiber filter paper is counted with a liquid ecintillation counter or a Yoounter. The test compound whose specific binding

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determine a product formed by a method suitable for determination of the product. Where an assay of the formation contained in the cells, the assay can be carried out with addition of an inhibitor of the degradation enzyme. In addition of an index compound of a cell stimulating activity (e.g., arachidonic acid, etc.) is difficult due to a degradation enzyme the culture is incubated for a given period of time, followed by extracting the cells or recovering the supernatant to taining the receptor protein are cultivated in a multi-well plate, etc. For carrying out screening, the culture medium is present invention by a known method or a commercially available determination kit. Specifically, first, the cells conas for cAMP production inhibitory activity or the like, the activity can be detected as the production inhibitory activity replaced with a fresh medium or a suitable buffer which does not have cytoloxicity. After addition of a test compound,

of cells whose basic production has been increased with forskolin, etc. protein are required. As cells expressing the receptor protein of the present invention, for example, naturally occurring cell strains containing the receptor protein of the present invention or the above-described recombinants expressing For carrying out the screening by measurement of a cell stimulating activity, cells expressing a suitable receptor

extracts, vegetable extracts, animal tissue extracts and the like and these compounds may be novel compounds or the receptor protein are preferred. Examples of the test compounds include peptides, proteins, non-peptide compounds, synthetic compounds, cell

a salt thereol, cells containing the receptor protein of the present invention, a cell membrane traction of cells containing invention comprises as an essential component the receptor protein or the partial peptide of the present invention or The kit for screening for a compound which alters binding of a ligand to the receptor protein or its salt of the present

20 the receptor protein of the present invention, or the like. Examples of the screening kit of the present invention include as follows

(1) Reagent for screening

25 (Gibco). The buffer is sterilized by filtration through a filter of 0.45 μm in pore diameter and then stored at 4°C or (a) Measurement buffer and washing buffer is can be prepare when it is used A buffer obtained by addition of 0.05% of bovine serum albumin (Sigma) to Hanks' balanced selt solution

(b) Standard of G-protein coupled receptor protein

 5×10^5 cells/well in a 12-well plate and cultivated at 37°C for 2 days in 5% CO $_2$ -95% air to obtain a standard of CHO cells expressing the receptor protein of the present invention are subjected to passage in an amount of

(c) Labeled ligand An aqueous solution of the labeled test compound is stored at 4°C or -20°C and, when it is used, it is diluted A ligand labeled with a commercial available [3H], [125], [14C], [35S], etc. or another suitable label.

to 1 µM with the measurement buffer. (d) Ligand standard solution

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mM and stored at -20°C. A ligand is dissolved in PBS containing 0.1% bovine serum albumin (Sigma) at the final concentration of 1

(2) Measurement

ml of the measurement buffer and then 490 µl of the same buffer is added to the respective wells. (a) The receptor protein expressing CHO cells cultivated in a 12-well tissue culture plate is washed twice with 1

ŝ (b) 5 μ l of a 10-3 to 10-10 M solution of a test compound is added and then 5 μ l of the labeled ligand is added. They are reacted at room temperature for one hour. In order to evaluate a non-specific binding amount, 5 µl 10-3 M

(c) The reaction mixture is removed and the wells are washed three times with 1 ml of the washing buffer. The eled ligand bound to the cells is dissolved with 0.2 N NaOH-1% SDS and mixed with 4 ml of liquid scintillator

(d) Radioactivity is measured by a liquid scintillation counter (Beckman). Percent Maximum Binding (PMB) is calculated by the equation [1]: A (Wako Pure Chemical Industries, Ltd.).

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PMB={(B-NSB)/(B₀-NSB)] x 100

non-specific binding, and B_0 is a maximum binding value. wherein PMB is Percent Maximum Sinding, B is a value obtained with addition of a specimen, NSB is a value of

The compound or its salt obtained by using the ecreening method of the screening kit of the present invention alters binding of a ligand to the receptor protein or its salt of the present invention. Specifically, it is a compound or a salt thereof which binds to the receptor protein of the present invention and a exhibits cell stimulating activity mediated by the receptor prolein (i.e., so-called an agonist to the receptor protein of the present invention) or which binds to the receptor protein of the present invention but does not exhibit the cell stimulating activity (i.e., so-called an anlagonist of the present invention).

Examples of the compounds include peptides, proteins, non-peptide compounds, synthetic compounds, fermented products and the like and they may be novel compounds or known compounds.

Since the agonist to the receptor protein of the present invention has the same physiological activity as that of a ligand to the receptor protein, it is useful as an active component of a safe and low toxic pharmaceutical composition having the ligand activity

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On the other hand, since the antagonist to the receptor protein of the present invention can inhibit the physiological activity of a figand to the receptor protein, it is useful as an active component of a sale and low toxic pharmaceutical

If the compound or its salt obtained by the screening method or the screening kit of the present invention is used for a pharmaceutical composition as described above, any conventional manner can be employed. For example, the compound or its salt of the present invention can be used in the form of tablets, if necessary, providing sugar coating, capsules, elixirs, microcapsules, etc. for oral administration, or in the form of injectable preparations such as aseptic solutions or suspensions in water or other pharmaceutically acceptable solutions for parenteral administration. A pharmaceutical composition in a unit dosage form can be prepared by mixing the compound or its satt of the present invention with, for example, one or more pharmaceutically acceptable carriers, flavors, excipients, vehicles, presenv atives, stabilizers, binders, etc. according to generally acceptable manner. The effective component is contained in the composition in such an amount that a dose in the intended desired range can be obtained. composition for inhibiting the ligand activity.

Examples of additives to be mixed in tablets, caupsels, etc. include binders such as galatin, corn starch, tragacanth gum and gum arabic, excipients such as crystalline cellulose, swelling agents such as com starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweetenings such as sucrose, lactose and saccharin, flavors such as peppermint, akamono oil and cherry, and the like. In case of a capsule dosage unit form, in addition to the above components, it can contain a liquid carrier such as fat or olf. An injectable asspite composition can be prepared according to a conventional manner, for example, by dissolving or suspending the active component in a vehicle such as injectable water and a natural vegetable oil such as sesame oil, coconut oil, etc. Examples of injectable aqueous solution include physiological saline; isotonic solutions containing glucose and other adjuvants (e.g., D-sorbitol, D-mannitol, sodium chloride, etc.) and suitable dissolution aids, for example, alcohols (e.g., ethanol), potyalcohols (e.g., As an oily solution, for example, sesame oil, soybean oil, etc. can be used and a dissolution aid such as benzyl benzoate buller, sodium acetate buller), smoothing agents (e.g., benzalkonium chloride, procaine hydrochloride, etc.), stabilizers propylene glycol, polyeitrylene glycol), nonionic surfaciants (e.g., Polysorbaie 80™, HCO-50) may be further added. or benzyl akchol, etc. can be further added. The composition can further contain, for example, buffers (e.g., phosphate (e.g., human serum albumin, polyethylane glycol, etc.), preservatives (e.g., benzyl alcohol, phenol, etc.), anticxidants, and the like. The injectable preparation thus produced is normally lilled in an appropriate ampoule.

Since the pharmaceutical composition thus obtained is safe and low loxic, il can be administer to a human being and another mammal (e.g., rat, rabbit, sheep, pig, cattle, cat, dog, monkey, etc.)

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Although the amount of the compound or its salt of the present invention to be administered is varied according to particular subjects, internal organs to be treated, symptoms, routes of administration, etc. in general, for oral administration to an adult human being (as 60 kg body weight), the DNA is administered in an amount of about 0.1 mg/day to about 100 mg/day, prelerably about 1,0 mg/day to about 50 mg/day, more preferably about 1,0 mg to about 20 mg For parenteral administration to an adult human being (as 60 kg body weight), it is advantageous to administer the composition in the form of an injectable preparation in an amount of about 0.01 mg/day to about 30 mg/day, preferably about 0.1 mg/day to about 20 mg/day, more preferably about 0.1 mg/day to about 10 mg/day, though the single dosage is varied according to particular subjects, internal organs to be treated, symptoms, routes of administration, etc. As for other animals, the composition can be administered in the above amount with converting it into that for the body weight of 60 kg

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(VI) Preparation of antibody or antiserum against the G-protein coupled receptor protein, the partial peptide or salts

peptide or their salts of the present invention can be prepared by using the receptor protein or the partial peptide of the present invention or a salt thereof as the antigen according to a conventional antibody or antiserum preparation An antibody (e.g., monoclonal antibody, polyclonal antibody) or antiserum against the receptor protein, the partial process. For example, a monoclonal antibody can be prepared as follows.

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Preparation of monoclonal antibody

(a) Preparation of monoclonal antibody producer cells

viated to the receptor protein, etc.) as such or together with a suitable carrier or diluent is administered to a site of a The receptor protein or the partial peptide of the present invention or a salt thereof (hereinatter sometimes abbremammal which permits the antibody production. For enhancing the antibody production capability, complete Freund's adjuvant or incomplete Freund's adjuvant may be administered. Normally, the receptor, etc. is administered once every 3 weeks to 6 weeks, in total, about 2 to about 10 times. The mammal to be used include monkey, rabbit, dog, guinea plg. mouse, rat, sheep, goat, chicken and the like and mouse or rat is preferred. 2

lected from warm blood animals immunized with the antigen, for example, rat and, 2 days to 5 days after the final antiserum can be carried out, for example, by reacting the labeled receptor protein, etc. as described hereinatter and For preparing monoclonal antibody producer cells, an individual whose antibody titer has been confirmed is seimmunization, its spleen or lymph node is collected and antibody producer cells contained therein are fused with myeloma cells to prepare the desired monoclonal antibody producer hybridoma. Measurement of the antibody titer in an an antiserum and then measuring the activity of the labeling agent bound to the antibody. The cell fusion can be carried out according to a known method, for example, the method described by Koehler and Milstein, Nature, 256, 495 (1975). As a fusion promoter, for example, potyethylene glycol (PEG) or Sendal virus (HVJ), preferably PEG can be used 35

Examples of myeloma cells include NS-1, P3U1, SP2/0, AP-1 and the like and P3U1 is preferred. The proportion of the number of antibody producer cells (spleen cells) and the number of myeloma cells to be used is preferably about Cell fusion can be carried out officiently by incubating a mixture of both cells at about 20°C to about 40°C, preferably l : 1 to about 20 : 1 and PEG (preterably PEG 1000-PEG 6000) is added in concentration of about 10% to about 80% about 30°C to about 37°C for about 1 minute to about 10 minutes. 20

Various methods can be used for screening for a hybridoma producing the antibody against the receptor protein, etc. For example, there may be mentioned a method wherein a supernatant of the hybridoma is added to a solid phase (e.g., microplate) to which the receptor protein antibody is adsorbed directly or together with a carrier and then an antimmunoglobulin antibody (if cells of a mouse is used in cell fusion, anti-mouse immunoglobulin antibody is used) of Protein A labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the receptor protein, etc. bound to the solid phase, and a method wherein a supernalant of the hybridoma is added to a labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the receptor solid phase to which an anti-immunoglobulin antibody or Protein A is adsorbed and then the receptor protein, etc., protein, etc. bound to the solid phase. 52 8

Selection of the monoclonal antibody can be carried out according to a per se known method or its modification. Normally, a medium for animal cells to which HAT (hypoxanthine, aminopterin, thymidine) are added is employed. Any selection and growth medium can be employed in so far as the hybridoma can grow. For example, RPMI 1640 medium containing 1% to 20%, preferably 10% to 20% fetal bovine serum, GIT medium containing 1% to 10% fetal bovine sorum (Wako Pure Chemical Industries, Ltd.), a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Selyaku) and the like can be used. Normally, the cultivation is carried out at 20°C to 40°C, preferably 37°C for about 5 days to about 3 weeks, preferably 1 week to 2 weeks under about 5% CO₂ gas. The antibody titer of the supernatant of a hybridoma culture can be measured according to the same manner as described above with respect to the antibody titer of the anti-G-protein coupled receptor in the antiserum.

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(b) Purification of monoclonal antibody

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Separation and purification of a monoclonal antibody against the receptor protein, etc. (hereinatter sometimes referred to as the anti-receptor protein monoclonal antibody) can be carried out according the same manner as those of conventional polyctonal antibodies such as separation and purification of immunoglobulins, for example, salting-out, alcoholic pracipitation, isoalactric point pracipitation, elactrophorasis, adsorption and desorption with ion exchangers (e.g., DEAE), ultracentrifugation, gel filtration, or a specific purification method wherein only an antibody is collected with an active adsorbent such as an antigen-binding solid phase, Protein A or Protein G and dissociating the binding to obtain the antibody. 20

The antibody against the receptor protein, etc. of the present invention prepared by the above (a) and (b) can specifically recognize the receptor protein, etc. of the present invention and therefore it can be used in a quantitative determination of the receptor protein, etc. of the present invention in a specimen, particularly, a quantitative determination by a sandwich immunoassay. That is, the present invention also provides:

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(a) a method for determination of the receptor protein or its salt of the present invention in a specimen fluid which comprises reacting an antibody against the receptor protein or the partial peptide of the present invention, the

specimen fluid and the labeled receptor protein or the labeled partial peptide competitively and measuring the proportion of the labeled receptor protein or the labeled partial peptide bound to the antibody, and (b) a method for determination of the receptor protein or its salt of the present invention in a specimen fluid which comprises reacting the specimen fluid, an antibody insubbilized on a carrier and a labeled antibody simultaneously or continuously, and then measuring the activity of the labeling agent on the insolubilized carrier, one antibody being that recognizing the N-terminal of the receptor protein of the present invention and the other antibody being

that reacting with the C-terminal of the receptor protein of the present invention.

In addition to the determination of the receptor protein or its salt of the present invention, the anti-eceptor protein monoclonal antibody recognizing the receptor protein of the present invention, etc. can be used for detection by hisnotogical stains and the like. For these purposes, the antibody molecular as such can be used or feath of Fab
fraction of the antibody molecule can also be used. A method for determination using an antibody against the receptor
fraction of the antibody molecule can also be used. A method for determination method can be used in as protein, atc. of the present invention is not specifically limited and any determination method can be used in as far as an amount of an antigen, antibody or antibody-antigen corresponding to an amount of an antigen in a fluid to be dean amount of an antigen, antibody or antibody-antigen corresponding to an amount of an antigen curve prepared by
minied can be detected by a chremical or physical means and calculated based on a calibration curve prepared by
sing standard solutions containing known amounts of the antigen. For example, nephelometry, competitive method,
immunormatic method and sandwich method are suitably employed. In particular, in view of sensitivity, specificily and
the like, a sandwich method as described hereinafter is preferred.

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As a labeling agent used in a determination method using a labeled reagent, radioisotopes, enzymas, fluorescent materials, turninous materials and the like can be used. Examples of radioisotopes include [125], [191], [191], [140] and the like. As the above enzymes, that having good stability and high specific activities is preferred and, for example, there are by-galactosidase, p-glucosidase, alkaline phosphatase, peroxidase, materials dehydrotogenase and the like. As the fluorescent materials, for example, there are functorescentaline, fluorescent insothicozyanate and the like. As the functions there are function, biolin-avidin system can materials, there are functions.

For insolubilization of an antigen or antibody, physical adsorption can be used or, normally, a method using a For insolubilization of an antigen or antibody, physical adsorption can be used. Examples of the carrier include chemical bond for insolubilizing or immobilizing a protein or an enzyme can be used. Examples of the carrier include chemical bond for insolubilization or immobilizing a protein or an enzyme can be used. Examples of the carrier include insoluble polysaccharides such as agarose, dextran, cellulose and the like, synthetic resins such as polystyrene, polysaccharides, silicone and the like, glass and the like.

In a sandwich method, a specimen fluid to be tested is reacted with an insolublized anti-receptor protein antibody (secondary reaction), followed by meast (primary creation) and further reacting a labeled anti-receptor protein antibody (secondary reaction), followed by meast uring the labeling agent on the insoluble carrier to determine the amount of the receptor protein of the present invention in the specimen. The order of the primary and secondary reactions can be reversed and they can be carried out simultaneously or separately at different times. The above-described labeling agent and insolubilization can be applied simultaneously or separately at different times. The above-described labeling agent and insolubilization can be applied to this method. In addition, in an immunoassay by a sandwich method, an antibody to be used as the solid phase antibody or labeled antibody is not necessary one kind of antibodies and, in order to improve measuring sensitivity, atc., a mixture of two or more kinds of antibodies can be used.

In the method for determination of the receptor protein, etc. by the sandwich method of the present invention, preferably, the antibodies against the receptor protein, etc. used in the primary and secondary reactions are those preferably, the antibodies against the receptor protein. For example, when the antibody used in the secondary reactions having different binding sites for the receptor protein. For example, when the antibodies used in the primary reaction is that the cognizing the C-terminal region of the receptor protein, the antibodies used in the primary reaction is that cognizing an region other than the C-terminal region, for example, the N-terminal region.

The antibody against the receptor protein, etc. of the present invention can also be used for a measuring system.

The antibody against the receptor protein, etc. of the present invention can also be used for a measuring system other than a sandwich method, for example, a competitive method, immunometric method, nephelometry and the like. In a competitive method, an antigen in a speciment fluid and a labeled antigen are cated with the antibody competitively and, after separation of the unreacted babeled antigen (F) from the labeled antigen bound to the antibody competitively and, after separation of the labeling agent of either B or F to determine the amount of the antigen in the specimen fluid. In this the amount of the labeling agent of either B or F to determine the amount of the antigen in the specimen fluid. In this the amount of the labeling agent of either B or F to determine the amount of the antigen in the specimen fluid. In this the amount of the labeling agent of either B or F to determine the amount of the antigen in the specimen fluid. In this the amount of the labeling agent of either B or F to determine the amount of the antigen in the specimen fluid. In this the amount of the labeling agent of either B or F to determine the amount of the antigen in the specimen fluid. In this the amount of the labeling agent of either B or F to determine the amount of the antibody is used as the first antibody and an immobilized solid phase antibody is used as the second antibody and an immobilized solid phase antibody is used as the second antibody and an immobilized solid phase antibody is used as the second antibody and an immobilized solid phase antibody is used as the second antibody and an immobilized solid phase antibody is used as the second antibody and an immobilized solid phase antibody is used as the second antibody and an immobilized solid phase antibody is used as the second antibody and an immobilized solid phase antibody and an immobilized solid phase antibody.

in the immunometric method, an antigen in a specimen fluid to be tested and an immobilized solid phase antigen in the immunometric method, an antigen in a specimen fluid to be tested then the solid phase is separated from the are reacted with a give amount of a labeled antibody, competitively and then the access amount of a labeled itguid phase. Alternatively, an antigen in a specimen fluid to be tested is reacted with an excess amount of a labeled antibody and an immobilized solid phase antigen is added to permit the unreacted tabeled antibody to bind to the solid phase from the liquid phase. Then, the amount of the labeling agent of phase, followed by the separation of the solid phase from the liquid phase. Then, the amount of the labeling agent of either phase is measured to determine the amount of the antigen in the specimen fluid.

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In nephelometry, an antigen-antibody reaction is carried out in a gel or solution and the amount of an insoluble precipitate formed is measured. Even when the amount of an antigen in a specimen fluid to be tested is small and the amount of a precipitate formed is small, laser nephelometry wherein diffusion of laser is utilized can be suitably emanded to the precipitate formed is small, laser nephelometry wherein diffusion of laser is utilized can be suitably emanded.

when employing these immunoassay methods in the determination method of the present invention, to set any special conditions, procedures and the like is not required. That is, the determination system of the receptor protein or its saft of the present invention can be constituted based on conventional conditions and procedures in respective methods together with conventional artisan's technical consideration. As for details of these general technical means, methods together with conventional artisan's technical consideration. As for details of these general technical means, reference can be made to various reviews, texts and the like, for example, Hiroshi rite, Ed., Radioimmunoassay, Kodan-sha (1979); Eizi lahikawa et al., Ed., Enzyme immunoassay, Igaku-shoin (1987); heithod in Enzymology, Vol. 70, Immunochemical Techniques (Part Al), Academic Press; bid., Vol. 73, Immunochemical Techniques (Part Al), Academic Press; bid., Vol. 73, Immunochemical Techniques (Part C), bid., Vol. 94, Immunochemical Techniques (Part C), bid., Vol. 94, Immunochemical Techniques (Part C), bid., Vol. 92, Immunochemical Techniques (Part E), Monoclonal Antibodies and General Immunocassays Methods); bid. 15

Vol. 121, Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies and the like.

As described hereinabove, the receptor protein or its salt of the present invention can be determined at high sensitivity by using an antibody against the receptor protein, etc. of the present invention.

In the specification and drawings, the abbreviations of bases, amino acids and the like are those according to IUPAC-IUB Commission on Biochemical Nomenclature or those conventionally used in the art. The examples are as follows. When the amino acid has an optical isomer, the amino acid is L-isomer unless otherwise stated.

23 g မ္ပ 55 8 dATP: dTTP: mRNA CDNA: dGTP ATP: GC TP Ž E SDS: Cys: ₹ Fen: Val: ₽ His: 7 ŢŸ. Asp: guanine thymine complementary deoxyribonucleic acid messenger ribonucleic acid deaxyribonucleic acid deoxyadenosine triphosphate serine glycine ethylenediaminetetraacetic acid adenosine triphosphate deoxycytidine triphosphate deoxyguanosine triphosphate deoxythymidine triphosphate ribonucleic acid cytosine cysteine threonine isoleucine leucine valine alanine enzyme immunoassay sodium dodecylsulfate asparagine glutamine proline arginine ysine glutamic acid tyrosine aspartic acid methionine tryptophan phenylalanine histidine

pyroglutamic acid pGlu:

methyl group ethyl group ₩

butyl group .: B

thiazolidin-4(R)-carboxamide group phenyl group 유 2

The sequences in the Sequence Listing of the present specification are as follows.

SEO ID NO: 1 5

This represents an amino acid sequence of the G-protein coupled receptor protein derived from a human brain.

SEC ID NO: 2

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This represents an amino acid sequence of the G-protein coupled receptor protein derived from a human brain, which is a variant of SEQ ID NO: 1 having additional 61 amino acids at the N-terminal thereof.

SEQ ID NO: 3 20

This represents a nucleotide sequence of DNA encoding the G-protein coupled receptor protein derived from a human brain having the amino acid sequence represented by SEQ ID NO: 1.

SEG ID NO: 4

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This represents a nucleotide sequence of DNA encoding the G-protein coupled receptor protein derived from a human brain having the amino acid sequence represented by SEQ ID NO: 2, which is a variant of SEQ ID NO: 3 having additional 183 bases at the 5'-terminal thereof.

SEQ ID NO: 5 8

A nucleotide sequence of EST which has been registered with a data base (NCBI abEST) under the accession number of T08099.

SEQ ID NO: 6 ક્ષ

A nucleotide sequence of EST which has been registered with a data base (NCBI abEST) under the accession umber of T27053.

SEQ ID NO: 7 5

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEQ ID NO: 8 â

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEC ID NO: 9 જ

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEQ ID NO: 10 જ

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

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SEC ID NO: 11

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein

coupled receptor protein of the present invention.

SEG ID NO: 12

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEQ ID NO: 13

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This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein

coupled receptor protein of the present invention.

SEQ ID NO: 14

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This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEQ ID NO: 15

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein

National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science & Technology Ministry The transformant Escherichia coli HB101/pHEBF2 obtained in Example 1 hereinafter has been deposited with of International Trade & Industry (1-3, Higasi 1-chome, Tsukubashi Ibaraki, 305 Japan) according to the Budapest Treaty under the accession number of FERM BP-5724 since October 25, 1996 and also deposited with Institute for Fermentation Osaka (IFO, 17-85, Juso-honmachi 2-chome Yodogawa-ku, Osaka, 532 Japan) under accession number coupled receptor protein of the present invention. 52

The following examples further illustrate the present invention in detail but are not to be construed to limit the scope of IFO 16044 since October 21, 1996. thereof

Example 1

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Obtaining cDNA of entire translated region of receptor protein from human poly(A)*RNA and sequence analysis of its nucleotide sequence

In order to obtain cDNA of the translated region about the C-terminal of the receptor protein encoded by the known nucleotide sequences, accession numbers of T08099 (SEQ ID NO: 5) and T27053 (SEQ ID NO: 6), 3°FACE (1) Obtaining cDNA of translated region about C-terminal of the receptor protein from human fetal brain poly (A)*RBNA by 3' RACE (Rapid Amplification of cDNA End) method and sequence analysis of its nucleotide sequence

First, the following two primers were synthesized based on the known nucleotide sequences. method was carried out by using human fetal brain poly(A)+RNA as a PCR template.

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B1: (SEQ ID NO: 7)

5'-AAGTTGGCTGTCATCTGGGTGGGCTC-3'

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B2: (SEQ ID NO: 8)

5'-reagcrectgergregeageregeage-3'

Then, a PCR template of 3PACE method was prepared from 1 µg human tatal poly(A)*RNA (Clontech) by using 3PACE kit (Gibco BRL). The first PCR was carried out using the primer attached to 3PACE kit and B1 primer. The conditions were 30 seconds at 95°C, 60 seconds at 65°C and 180 seconds at 72°C, for 35 cycles and

cloning kit (Invitrogen) and transferred into E. coli JM109. As a result of sequence analysis, it was found that the μl of the first PCR mixture as the template under the same conditions for 35 cycles except that B2 primer was used Ex Taq (Takara Shuzo) was used as the DNA polymerase. The second PCR reaction was carried out by using 1 (2) Obtaining cDNA of translated region about N4erminal of the receptor protein from human fetal brain poly amplified band had the C-terminal region of the above-described known nucleotide sequences. instead of B1 primer. After electrophoresis, a band formed of 1.5 kb was recovered, subcloned by using a TA

(A)+RNA by 5'RACE method (marathon method) and sequence analysis of its nucleotide sequence above-described known nuclectide sequences, S'RACE method was carried out by using human fetal brain poly In order to obtain cDNA of the translated region about the N-terminal of the receptor protein encoded by the

(A)+ RNA as a PCR template. First, the following two primers were synthesized based on the above-described known nucleotide sequences

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5'-CATGCGGGCGTTCTGGTAGGTCATCAC-3' B8: (SEQ ID NO: 9)

B9: (SEQ ID NO: 10

5'-GAAGAGGATGGGCAGGCAGAAGTAGCAG-3'

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cording to the manual of SPACE kit (Clontech). The first PCR was carried out using the primer attached to SPACE of this amino acid sequence. by the nucleotide sequence of from the 625th base (ATG; Met) to the 2067th base (TGC; Cys) as shown by Fig. (1). The presence of the 7-transmembrane receptor protein comprising 481 amino acids (SEQID NO: 1) encoded a TA cloning kit (Invitragen), and sequence analysis was carried out according to the same manner as in the above instead of B9 primer. After electrophoresis, a band formed of about 1 kb was recovered and subcloned by using 50-fold dilution of the first PCR mixture as the template under the same conditions except that B8 primer was used 98°C and 180 seconds at 70°C, for 5 cycles; and 10 seconds at 98°C and 180 seconds at 88°C, for 35 cycles. Ex kit and B9 primer. The conditions were 10 seconds at 98°C and 180 seconds at 72°C, for 5 cycles; 10 seconds at Taq (Takara Shuzo) was used as the DNA polymerase. The second PCR reaction was carried out by using 1 μl of I has been confirmed based on the results of the above (1) and (2). Fig. 2 shows the result of hydrophobic plotting . Then, a PCR template of 5'RACE method was prepared from 1 µg human tatal poly(A)+RNA (Clontech) ac-

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(3) The N-terminal side of the above receptor protein was further examined and 5'HACE method at the 5'side was further carried out to determine a transcription initiation codon.

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above (2) The following two primers were synthesized based on the translation region of the receptor protein obtained in the



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B11: (SEQ ID NO: 11)

5'-ATGAAGGGCACGGCACGACAAGAAACG-3'

B12: (SEQ ID NO: 12)

5'-ATGACAATAGGGAGGCAGAAAAAGAGG-3'

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primer B12 instead of the primer B8, respectively to amplify the templates derived from the poly(A)+RNAs of the above as described in the above (2), twice PCR were carried out by using the primer B11 instead of the primer B9 and the brain poly(A)+RNA (Clontech) or human cerebellum poly(A)+RNA (Nippon gene). Then, according to the same manner and then subjected to sequence analysis. internal organs. After electrophoresis of the reaction product, a band formed was recovered, subcloned by TA cloning According to the same manner as in the above (2), a PCR template was prepared by using the above human fetal

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As a result, the sequence obtained by combining the above (1) to (3) had the nucleotide sequence (SEQ ID NO

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encoded the 7-transmembrane receptor protein comprising 542 amino acids (SEQ ID: NO 2). The result of the hydro-4) of from the 442nd base (ATC; Met) to the 2067th base (TCG; Cys) as shown in Fig. 3 and was confirmed that it phobic plotting of this sequence is shown in Fig. 4

The following primers were synthesized based on this sequence.

HEF: (SEQ ID NO: 13)

5'-GTCGACGAGATGTGTGAGGGCAGCAAAGAGTGC-3'

HER-1: (SEQ ID NO: 14)

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5'-TACTGGGGCCTCAGCAAGGTGTGCCCAG-3'

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E. coll HB101 to obtain the transformant, E. coll HB101/pHEBF2. The DNA contained in the plasmid pHEBF2 has the fetal brain cDNA library. After subcloning in E. coli, a clone without any PCR error was selected and transformed into nucleotide sequence rapresented by SEQ ID NO: 4 (Fig. 3) and the nucleotide sequence represented by SEQ ID NO: PCR was carried out by using these two primers to amplify the coding region for the receptor protein from human

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seconds at 68°C to amplify the coding region, the cDNA of the receptor protein as shown by Fig. 3 was obtained from 3 is contained therein. When PCR was carried out by using the above two primers under the conditions of 30 seconds at 95°C and 90

25 In order to obtain the cDNA of the receptor protein form adult brain, HEF-2 primer was synthesized instead of HEF

fetal brain

HEF-2: (SEQ ID NO: 15)

5'-GTCGACTGGCTGTCTCCTGCTCATCCAGCCAT-3'

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receptor protein shown in Fig. 3 by 61 amino acids. receptor protein shown in Fig. 1 was obtained. The N-terminal of this receptor protein was shorter than that of the When adult brain poly(A)+RNA was amplified by using the primers HEF-2 and HEF-1, the cDNA encoding the

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brain. On the other hand, cDNA encoding the receptor protein shown by Fig. 3 is obtained from fetal brain and the presence of the long chain receptor protein shown by Fig. 3 has also been confirmed. In view of these results, it has been found that the receptor protein shown by Fig. 1 is predominantly expressed in adult initiation of translation just before the translation initiation codon and a defined signal sequence in the N-terminal region. The receptor protein shown in Fig. 1 has a consensus sequence called as Cossack sequence which indicates

Example 2

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Confirmation of expression specificity in various tissues

Ġ the plasmid pHEBF2 obtained in Example 1 as a probe. Northern blot was carried out by using cDNA encoding the receptor protein of the present invention contained in

of the filter to light was made as -80°C for one week. As shown in Fig. 5, it has been found that this receptor protein ization was carried out by using human MTN Blot (Ciontech) and according to the manual attached thereto. Exposure The cDNA was labeled with Amarsham's multiprime kit and [22P]dCTP according to the manual of the kit. Hybrid

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coding them of the present invention can be used (a) for determination of ligands, (b) for obtaining antibodies and of drug design based on comparison of structurally analogous ligand receptors, (1) as reagents for preparing probes in gene diagnoses, PCR primers, etc., (g) drugs for gene prophylactic and therapy, and the like. binding assay systems and screening for drug candidate compounds by using the expression systems, (e) for practice antisera, (c) for construction of recombinant receptor protein expression systems, (d) for development of receptor mRNA is specifically expressed in brain. As described hereinabove, the G-protein coupled receptor protein, its partial peptide or their satte and DNAs en

SEQUENCE LISTING

stries Ltd

(ii) TITLE OF THE INVENTION: Novel Human G-Protein Coupled Receptor Protein and its DNA

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(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual Property
(B) STBEET, Tang Man Universely

(B) STREET: Two New Horizons Court (C) CITY: Brentford

(E) COUNTRY: United Kingdom

(D) COUNTY: Middlesex

(F) POST CODE: TW8 9EP

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette (B) COMPUTER: IBM Compatible

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(vi) CURRENT APPLICATON DATA: (A) APPLICATION NUMBER:

(A) APPLICATION NUMBEI (B) FILING DATE:

(C) CLASSIFICATION

(vii) PRIOR APPLICATION DATA: (A) APPLICATION NUMBER: 28623/1996

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(A) NAME: CONNELL, Anthony Christopher (B) GENERAL AUTHORISATION NUMBER:

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(2) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 481
(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

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(ii) MOLECULAR TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Met Arg Trp Leu Trp Pro Leu Ala Val Ser Leu Ala Val Ile Leu Ala 1 5 10 Val Gly Leu Ser Arg Val Ser Gly Gly Ala Pro Leu His Leu Gly Arg 20 25 30 His Arg Ala Glu Thr Gln Glu Gln Gln Ser Arg Ser Lys Arg Gly Thr 35 40 45 Glu Asp Glu Glu Ala Lys Gly Val Gln Gln Tyr Val Pro Glu Glu Trp 50 55 60

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Ala Giu Tyr Pro Arg Pro Ile His Pro Ala Giy Leu Gin Pro Thr Lys
65 70 75 80
Pro Leu Val Ala Thr Ser Pro Asn Pro Asp Lys Asp Gly Gly Thr Pro
85 90 95

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Asp Ser Gly Glu Leu Arg Gly Asn Leu Thr Gly Ala Pro Gly Gln 100 105

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Arg Leu Gin Ile Gin Asn Pro Leu Tyr Pro Val Thr Giu Ser Ser Tyr 115 120 125

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Ser Ala Tyr Ala Ile Met Leu Leu Ala Leu Val Val Phe Ala Val Gly 130 135 140 lle Val Gly Asn Leu Ser Val Met Cys lle Val Trp His Ser Tyr Tyr 145 150 Leu Lys Ser Ala Trp Asn Ser lle Leu Ala Ser Leu Trp Asp

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	170	
;	175	

Phe Leu Val Leu Phe Phe Cys Leu Pro Ile Val Ile Phe Asn Glu Ile
180 185 190

Thr Lys Gln Arg Leu Leu Gly Asp Val Ser Cys Arg Ala Val Pro Phe
195 200 205

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Met Glu Val Ser Ser Leu Gly Val Thr Thr Phe Ser Leu Cys Ala Leu 210 215 220

Gly lle Asp Arg Phe His Val Ala Thr Ser Thr Leu Pro Lys Val Arg 225 230 235 240 Pro Ile Glu Arg Cys Gln Ser lle Leu Ala Lys Leu Ala Val Ile Trp 245 250 255

Val Gly Ser Met Thr Leu Ala Val Pro Glu Leu Leu Leu Trp Gln Leu 260 265 270

Ala Gln Glu Pro Ala Pro Thr Met Gly Thr Leu Asp Ser Cys lle Met 275 280 285

Lys Pro Ser Ala Ser Leu Pro Giu Ser Leu Tyr Ser Leu Val Met Thr 290 295 300

Tyr Gin Asn Ala Arg Met Trp Trp Tyr Phe Gly Cys Tyr Phe Cys Leu 305 310 315 320 Pro Ile Leu Phe Thr Val Thr Cys Gin Leu Val Thr Trp Arg Val Arg 325 330 335

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Gly Pro Pro Gly Arg Lys Ser Glu Cys Arg Ala Ser Lys His Glu Gin 340 345 350

Cys Glu Ser Gln Leu Asn Ser Thr Val Val Gly Leu Thr Val Val Tyr 355 360 365

Ala Phe Cys Thr Leu Pro Glu Asn Val Cys Asn Ile Val Val Ala Tyr 370 375 380

Leu Ser Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly Leu Ile 385 390 206

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Asn Gin Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val Leu Leu 405 410 415

Leu Cys Ile Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys Cys Cys 420 425 430

Cys Cys Cys Giu Giu Cys Giy Giy Ala Ser Giu Ala Ser Ala Ala 435 440 445

Asn Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser Ile Tyr 450 455 460

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Phe His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly Thr Pro 465 470 475 480 Cys

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(3) INFORMATION FOR SEQ ID NO: 2

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 542

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(B) TYPE: amino acid
(C) STRANDEDNESS

(C) STRANDEDNESS: (D) TOPOLOGY: linear

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(ii) MOLECULAR TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Cys Pro Ala Glu Gly Pro Ala Arg Pro Val Ala Gly Gly Trp Glu 1 5 10 15

Gly Gly Gln Ala Ser Asp Ala Arg Arg Leu Thr Gly Gly Gly Ser Ser 20 25 30

Arg Pro Ala Ala Ser Leu Glu Pro Ser Ser Trp Ala Pro Cys Thr His 35 40 45

Leu Leu Phe Leu Gly Trp Leu Ser Pro Ala His Pro Ala Met Arg Trp
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60
Leu Trp Pro Leu Ala Val Ser Leu Ala Val Ile Leu Ala Val Gly Leu
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Ser Arg Val Ser Gly Gly Ala Pro Leu His Leu Gly Arg His Arg Ala 85 90 95

Glu Thr Gln Glu Gln Ger Arg Ser Lys Arg Gly Thr Glu Asp Glu 100 105

Giu Ala Lys Gly Vál Gin Gin Tyr Val Pro Giu Giu Trp Ala Giu Tyr 115 120 125

5

Pro Arg Pro Ile His Pro Ala Gly Leu Gln Pro Thr Lys Pro Leu Val 130 135 Ala Thr Ser Pro Asn Pro Asp Lys Asp Gly Gly Thr Pro Asp Ser Gly
145 150 155 160
Gln Glu Leu Arg Gly Asn Leu Thr Gly Ala Pro Gly Gln Arg Leu Gln
165 170 175

lle Gln Asn Pro Leu Tyr Pro Val Thr Glu Ser Ser Tyr Ser Ala Tyr 180 185 190

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Ala IIe Met Leu Leu Ala Leu Val Val Phe Ala Val Gly IIe Val Gly 195 200 205

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Asn Leu Ser Val Met Cys Ile Val Trp His Ser Tyr Tyr Leu Lys Ser 210 215 Ala Trp Asn Ser lle Leu Ala Ser Leu Ala Leu Trp Asp Phe Leu Val 225 230 230 235 240 Leu Phe Phe Cys Leu Pro lle Val Ile Phe Asn Glu Ile Thr Lys Gln 245 250 Arg Leu Leu Gly Asp Val Ser Cys Arg Ala Val Pro Phe Met Glu Val 260 265 270

Ser Ser Leu Gly Vai Thr Thr Phe Ser Leu Cys Ala Leu Gly Ile Asp 275 280 285 Arg Phe His Val Ala Thr Ser Thr Leu Pro Lys Val Arg Pro Ile Glu 290 295

23

Arg Cys Gln Ser Ile Leu Ala Lys Leu Ala Val Ile Trp Val Gly Ser

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305 310 315 320 Met Thr Leu Ala Val Pro Glu Leu Leu Leu Trp Gln Leu Ala Gln Glu 325 330 335

Pro Ala Pro Thr Met Gly Thr Leu Asp Ser Cys Ile Met Lys Pro Ser 340 340 345 Ala Ser Leu Pro Glu Ser Leu Tyr Ser Leu Val Met Thr Tyr Gln Asn 355 360 365

6

Ala Arg Met Trp Trp Tyr Phe Gly Cys Tyr Phe Cys Leu Pro lle Leu 370 375 380
 Phe Thr Val Thr Cys Glu Leu Val Thr Trp Arg Val Arg Gly Pro Pro

 385
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 Gly Arg Lys Ser Glu Cys Arg Ala Ser Lys His Glu Gln Cys Glu Ser
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Gln Leu Asn Ser Thr Val Val Gly Leu Thr Val Val Tyr Ala Phe Cys 420 425 430 Thr Leu Pro Glu Asn Val Cys Asn Ile Val Val Ala Tyr Leu Ser Thr 435 440 445

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Giu Leu Thr Arg Gin Thr Leu Asp Leu Leu Gly Leu Ile Asn Gin Phe 450 . 455 460

Ser Thr Phe Phe Lys Gly Ala lie Thr Pro Val Leu Leu Leu Cys lie 465 470 475 480
Cys Arg Pro Leu Gly Gin Ala Phe Leu Asp Cys Cys Cys Cys Cys Cys 485 490 495

Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser Ala Asn Gly Ser 500 505 510

Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser Ile Tyr Phe His Lys 515 520 525

20

Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly Thr Pro Cys 530 535 540

(4) INFORMATION FOR SEQ ID NO: 3

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1443
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGCGGTGGC TGTGGCCCCT GGCTGTCTCT CTTGCTGTGA TTTTGGCTGT GGGGCTAAGC

AGGGTCTCTG GGGGTGCCCC CCTGCACCTG GGCAGGCACA GAGCCGAGAC CCAGGAGCAG

20

25 CAGAGCCGAT CCAAGAGGGG CACCGAGGAT GAGGAGGCCA AGGGCGTGCA GCAGTATGTG

CCTGAGGAGT GGGCGGAGTA CCCCCGGCCC ATTCACCCTG CTGGCCTGCA

GCCAACCAAG

CCCTTGGTGG CCACCAGCCC TAACCCCGAC AAGGATGGGG GCACCCCAGA CAGTGGGCAG 35 300

GAACTGAGGG GCAATCTGAC AGGGGCACCA GGGCAGAGGC
TACAGATCCA GAACCCCCTG
360

TATCCGGTGA CCGAGAGCTC CTACAGTGCC TATGCCATCA TGCTTCTGGC
GCTGGTGGTG

TITTGCGGTGG GCATTGTGGG CAACCTGTCG GTCATGTGCA TCGTGTGGCA CAGCTACTAC
480

CTGAAGAGCG CCTGGAACTC CATCCTTGCC AGCCTGGCCC TCTGGGATTT
 TCTGGTCCTC
 \$40

TTTTTCTGCC TCCCTATTGT CATCTTCAAC GAGATCACCA AGCAGAGGCT ACTGGGTGAC 600

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GTTTCTTGTC GTGCCGTGCC CTTCATGGAG GTCTCCTCTC TGGGAGTCAC GACTTTCAGC

660
CTCTGTGCCC TGGGCATTGA CCGCTTCCAC GTGGCCACCA GCACCCTGCC
CAAGGTGAGG
720

CCCATCGAGC GGTGCCAATC CATCCTGGCC AAGTTGGCTG TCATCTGGGT
GGGCTCCATG
780

ACGCTGGCTG TGCCTGAGCT CCTGCTGTGG CAGCTGGCAC AGGAGCCTGC
CCCCACCATG

840
GGCACCCTGG ACTCATGCAT CATGAAACCC TCAGCCAGCC TGCCCGAGTC
CCTGTATTCA

CTGGTGATGA CCTACCAGAA CGCCCGCATG TGGTGGTACT TTGGCTGCTA
CTTCTGCCTG

25 960
CCCATCCTCT TCACAGTCAC CTGCCAGCTG GTGACATGGC GGGTGCGAGG

CCCATCCTCT TCACAGTCAC CTGCCAGCTG GTGACATGGC GGGTGCGAGG CCCTCCAGGG 1020 AGGAAGTCAG AGTGCAGGGC CAGCAAGCAC GAGCAGTGTG

AGGAAGTCAG AGTGCAGGGC CAGCAAGCAC GAGCAGTGTG AGAGCCAGCT CAACAGCACC

STGGTGGGCC TGACCGTGGT CTACGCCTTC TGCACCCTCC CAGAGAACGT
CTGCAACATC
1140
GTGGTGGCCT ACCTCTCCAC CGAGCTGACC CGCCAGACCC TGGACCTCCT

40 GGGCCTCATC
1200
AACCAGTTCT CCACCTTCTT CAAGGGCGCC ATCACCCCAG TGCTGCTCCT
TTGCATCTGC

1260
AGGCCGCTGG GCCAGGCCTT CCTGGACTGC TGCTGCTGCT GCTGCTGTGA
GGAGTGCGGC

SO GGGGCTTCGG AGGCCTCTGC TGCCAATGGG TCGGACAACA AGCTCAAGAC
CGAGGTGTCC

TCTTCCATCT ACTTCCACAA GCCCAGGGAG TCACCCCCAC TCCTGCCCCT
65 GGGCACACCT

v3	1440 TGC 1443	CTGTATCCGG TGACCGAGAG CTCCTACAGT GCCTATGCCA TCATGCTTCT GGCGCTGGTG
	(5) INFORMATION FOR SEQ ID NO: 4	
01	(I) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1626 (B) TYPE: nucleic acid	980 TACCTGAAGA GCGCCTGGAA CTCCATCCTT GCCAGCCTGG CCCTCTGGGA TTTTCTGGTC
15	(C) STRANDEDNESS: double (D) TOPOLOGY: linear	720 CTCTTTTTCT GCCTCCCTAT TGTCATCTTC AACGAGATCA CCAAGCAGAG 15 GCTACTGGGT
	(ii) MOLECULAR TYPE: ¢DNA	780 GACGTTTCTT GTCGTGCCGT GCCCTTCATG GAGGTCTCCT CTCTGGGAGT
20	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:	20 CACGACTTTC
ž	ATGTGTCCAG CAGAGGGCCC TGCCCGGCCT GTGGCCGGAG GCTGGGAGGG AGGCAGGCG	
Q	AGO	25 YOU AGCCCATCG AGCGGTGCCA ATCCATCCTG GCCAAGTTGG CTGTCATCTG GGTGGGCTCC
90	AGCTCTTGGG CCCCCTGTAC TCACCTGCTC TTCCTGGGCT GGCTGTCTCC TGCTCATCCA	ACCCCCACC TGCCCCCACC TGCCCCCCACC TGCCCCCACC TGCCCCCACC TGCCCCCACC TGCCCCCACC TGCCCCCACC TGCCCCCACC TGCCCCCACC TGCCCCCACC TGCCCCCCACC TGCCCCCACCC TGCCCCCCACC TGCCCCCACCC TGCCCCCACCC TGCCCCCACCC TGCCCCCCACCC TGCCCCCCACCC TGCCCCCCACCC TGCCCCCCACCC TGCCCCCACCC TGCCCCCACCC TGCCCCCCACCC TGCCCCCCACCC TGCCCCCCACCC TGCCCCCCACCC TGCCCCCACCCCACCC TGCCCCCCACCCCACCCCACCCCACCCACCCACCCACCCA
ક્ષ	TOTGGGGCTA TGTGGGCTA TGTGGGCTA TGTGGGCTA TGTGGGCTA	1020 ATGGCACCC TGGACTCATG CATCATGAAA CCCTCAGCCA GCCTGCCCGA GTCCCTGTAT IDRO
\$	AGCCAGGGTCT CTGGGGTGC CCCCTGCAC CTGGGCAGGC ACAGAGCCGA GACCCAGGAG 300	TCACTGGTGA TGACCTACCA GAACGCCCGC ATGTGGTGGT ACTTTGGCTG CTACTTCTGC 40 1140
	CAGCAGAGCC GATCCAAGAG GGGCACCGAG GATGAGGAGG CCAAGGGCGT GCAGCAGTAT	CTGCCCATCC TCTTCACAGT CACCTGCCAG CTGGTGACAT GGCGGGTGCG AGGCCCTCCA
3	JOU GTGCCTGAGG AGTGGGGGA GTACCCCGG CCCATTCACC CTGCTGGCCT GCAGCCAACC	45 1200 GGGAGGAAGT CAGAGTGCAG GGCCAGCAAG CACGAGCAGT GTGAGAGCCA GCTCAACAGC 1760
98	AAGCCCTTGG TGGCCACCAG CCCTAACCCC GACAAGGATG GGGGCACCCC AGACAGTGGG 480	ACCGTGGTGG GCCTGACCGT GGTCTACGCC TTCTGCACCC TCCCAGAGAA CGTCTGCAAC .
53	540 CAGGAACTGA GGGGCAATCT GACAGGGGCA CCAGGGCAGA GGCTACAGAT CCAGAACCCC	33

ATCAACCAGT TCTCCACCTT CTTCAAGGGC GCCATCACCC CAGTGCTGCT ATCGTGGTGG CCTACCTCTC CACCGAGCTG ACCCGCCAGA CCCTGGACCT CCTGGGCCTC

CCTTTGCATC

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TGCAGGCCGC TGGGCCAGGC CTTCCTGGAC TGCTGCTGCT GCTGCTGCTG TGAGGAGTGC

GACCGAGGTG GGCGGGGCTT CGGAGGCCTC TGCTGCCAAT GGGTCGGACA ACAAGCTCAA

CCTGGGCACA TCCTCTTCCA TCTACTTCCA CAAGCCCAGG GAGTCACCCC CACTCCTGCC

CCTTGC

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(6) INFORMATION FOR SEQ ID NO: 5

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 426

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: double

(D) TOPOLOGY: lineau

(ii) MOLECULAR TYPE: cDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTGGCTGTGC TGCAATCCAT CCTGGCCAAG TTGGCTGTCA TCTGGGTGGG CTCCATGACG

ŝ ACCCTGGACT CTGAGCTCCT GCTGTGGCAG CTGGCACAGG AGCCTGCCCC CACCATGGGC

60

CATGCATCAT GAAACCCTCA GCCAGCCTGC CCGAGTCCCT GTATTCACTG GTGATGACCT

8

ACCAGAACGC CCGCATGTGG TGGTACTTTG GCTGCTACTT CTGCCTGCCC ATCCTCTTCA

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CAGTCACCTG CCAGCTGGTG ACATGGCGGG TGCGAGGCCC TCCAGGGAGG AAGTCAGAGT

GTGGGCCTGA CCGTGGTCTA CGGCTTTTTG CAACCTTCCA GAGAACGTTT GCAACATCGT GCAGGGCCAG CAAGCACGAG CAGTGTGAGA GCCAGCTCAA CAGCACCGTG

GGTGGGCTTA

70

CCTTTT

(7) INFORMATION FOR SEQ ID NO: 6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 248

8

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

25

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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ACATCGTGGT AACAAGGGCC GTGGTCTACG NCTTCTGCAC CCTCCCANAG AACGTCTGCA

GGCCTACCTC TCCACCGAGC TGACCCGCCA GNCCCTGGAC CTCCTGGGCC TCATCAACCA

TCTGCAGGCC GTTCTCCACC TTCTTCAAGG GCGCCATCAC CCCAGTGCTG CTCCTTTGCA

GCGGCGGGC GCTGGGCCAG GCCTTCCTGG ACTGCTGCTG CTGCTGCTGC TGTNAGGAGT

TTCGGAGG

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(8) INFORMATION FOR SEQ ID NO: 7

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

(ii) MOLECULAR TYPE: synthetic DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:	GAAGAGGATG GGCAGGCAGA AGTAGCAG 28	(i) SEQUENCE CHARACTERISTICS:	(A) LENG I H: 2/ (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11: ATGAAGGGCA CGGCACGACA AGAAACG 27		(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 (B) TYPE: nucleic acid (C) STRANDEDNESS: single	(D) TOPOLOGY: linear 35 (ii) MOLECULAR TYPE: synthetic DNA	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:	ATGACAATAG GGAGGCAGAA AAAGAGG 27	(14) INFORMATION FOR SEQ ID NO: 13	(a) LENGTH: 33 (b) TYPE: nucleic acid (c) STRANDEDNESS: single (d) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA
(B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7: AAGTTGGCTG TCATCTGGGT GGGCTC 26	(9) INFORMATION FOR SEQ ID NO: 8 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 28	(b) 117E: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8: TGAGCTCCTG CTGTGGCAGC TGGCACAG 28	(10) INFORMATION FOR SEQ ID NO: 9 (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27	(B) TYPE: nuclete acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:	CATGCGGGCG TTCTGGTAGG TCATCAC 27	(11) INFORMATION FOR SEQ ID NO: 10 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 28 (B) TYPE: nucleic acid (C) STRANDEDNESS: single	(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13: GTCGACGAGA TGTGTGAGGG CAGCAAAGAG TGC ដ

(i) SEQUENCE CHARACTERISTICS: (15) INFORMATION FOR SEQ ID NO: 14

(A) LENGTH: 28

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: synthetic DNA

TACTGGGGCC TCAGCAAGGT GTGCCCAG 28 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

8

(16) INFORMATION FOR SEQ ID NO: 15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32

8

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(ii) MOLECULAR TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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GTCGACTGGC TGTCTCCTGC TCATCCAGCC AT 32

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SEQUENCE LISTING

INFORMATION FOR SEQ ID NO: 1

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 481
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

5

(ii) MOLECULAR TYPE: peptide

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Glu Asp Glu Glu Ala Lys Gly Val Gln Gln Tyr Val Pro Glu Glu Trp His Arg Ala Glu Thr Gin Glu Gin Gin Ser Arg Ser Lys Arg Gly Thr Val Gly Leu Ser Arg Val Ser Gly Gly Ala Pro Leu His Leu Gly Arg Met Arg Trp Leu Trp Pro Leu Ala Val Ser Leu Ala Val Ile Leu Ala Asp Ser Gly Gln Glu Leu Arg Gly Asn Leu Thr Gly Ala Pro Gly Gln Pro Leu Val Ala Thr Ser Pro Asn Pro Asp Lys Asp Gly Gly Thr Pro Ala Glu Tyr Pro Arg Pro Ile His Pro Ala Gly Leu Gln Pro Thr Lys 65 S 35 90 20 70 55 6 105 8 75 8 110 85 15 80

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ä		/a1	140	Γŗ		Leu		116	•	Ars		Ser Leu Cys Ala	220	Leu		Leu		Leu		γsb	
/a1]		/81		Val.	155	Ser		Val		Cys		Phe		Thr	235	Lys		Leu		Ľer	
01,		18		116	•	A la	170	Ile		Ser		18.		Ser		Ala	250	Gla		Ę	
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Arg Leu Gin ile, Gin Asn Pro Leu Tyr Pro Val Thr Giu Ser Ser	120	Ser Ala Tyr Ala Ile Met Leu Leu Ala Leu Val Val Phe Ala Val	•	Cly Asn Leu Ser Val Met Cys Ile Val Trp His Ser Tyr		Ser Ala Trp Asa Ser Ile Leu Ala Ser Leu Ala Leu Trp Asp		Phe Leu Val Leu Phe Phe Cys Leu Pro Ile Val Ile Phe Asn Glu Ile		Lys Gln Arg Leu Leu Gly Asp Val Ser Cys Arg Ala Val	200	Glu Val Ser Ser Leu Gly Val Ihr Ihr Phe		Gly lle Asp Arg Phe His Val Ala Thr Ser Thr Leu Pro Lys Val Arg		Pro Ile Glu Arg Cys Gla Ser Ile Leu Ala Lys Leu Ala Val Ile Trp		Ser Met Ihr Leu Ala Val Pro Glu Leu Leu Leu		Ala Gin Giu Pro Ala Pro Ihr Met Giy Ihr Leu Asp Ser	280
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Asn Ala Arg Met Trp Irp Iyr Phe Gly Cys Iyr Phe 310 310 315 Leu Phe Thr Val Thr Cys Gln Leu Val Thr Irp Arg 320 325 Pro Gly Arg Lys Ser Glu Cys Arg Ala Ser Lys His 340 340 355 Gys Thr Leu Pro Glu Asn Val Cys Asn Ile Val Val 355 Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly 336 Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly 336 Thr Glu Leu Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 11e Cys Arg Pro Leu Gly Gly Ala Ile Thr Pro Val 405 12or Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 420 35or Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 455 12ys Pro Arg Glu Ser Pro Leu Leu Pro Leu Gly 415 12ys Pro Arg Glu Ser Pro Leu Leu Pro Leu Gly 415 12ys Pro Arg Glu Ser Pro Leu Leu Pro Leu Gly 415	Lys	Pro 290	Ser	Ala	Ser	Leu	Pro 295	G1 u	Ser	Leu	Tyr	Ser 300	Leu	Val	Het	•
110 Leu Phe Thr Val Thr Cys Gla Leu Val Thr Trp Arg 325 330 345 345 350 346 345 345 345 350 346 345 346	Iyr			Ala	Arg	Met				Phe				Phe	Cys	
11e Leu Phe Thr Val Thr Cys Gln Leu Val Thr Trp Arg 325 330 345 350 340 345 345 350 310 Ser Gln Leu Asn Ser Thr Val Val Gly Leu Thr Val 355 360 365 380 385 370 375 380 395 371 Ser Thr Phe Phe Lys Gly Ala Thr Pro Val 405 Gys Thr Phe Phe Lys Gly Ala Thr Pro Val 405 Gys Gys Gys Gys Gys Gys Gys Gys 436 436 440 440 445 437 438 440 440 445 448 440 445 440 445 450 450 455 450 Gys Gys Gys Gys Gys 418 Lys Pro Arg Glu Gys Gys Gys Gys 418 Lys Pro Arg Glu Gys Gys Gys Gys 419 Gys Gys Gys Gys Gys Gys Gys 410 Gys Gys Gys Gys Gys Gys Gys Gys 410 Gys Gys Gys Gys Gys Gys Gys Gys 410 Gys Gys Gys Gys Gys Gys Gys Gys Gys 410 Gys Gys Gys Gys Gys Gys Gys Gys 411 Gys Gys Gys Gys Gys Gys Gys Gys 412 Gys Gys Gys Gys Gys Gys Gys Gys 413 Gys Gys Gys Gys Gys Gys Gys Gys 414 Gys Gys Gys Gys Gys Gys Gys Gys 415 Gys Gys Gys Gys Gys Gys Gys Gys Gys 416 Gys Gys Gys Gys Gys Gys Gys Gys Gys 417 Gys Gys Gys Gys Gys Gys Gys Gys Gys 418 Gys 419 Gys 410 Gys	305					310					315					
Sac Sac Sac Clu Cys Arg Ala Ser Lys His 340	Pro			Phe	Į.	Val	Thr		G1 a		Val	Thr		Ar 8		
Pro Pro Gly Arg Lys Ser Glu Cys Arg Ala Ser Lys His 340 Glu Ser Gln Leu Asn Ser Thr Val Val Gly Leu Thr Val 355 Bhe Cys Thr Leu Pro Glu Asn Val Cys Asn Ile Val Val 337 Ser Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly 337 Gln Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 Cys Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 420 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Glu Ala Ser 440 445 445 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Glu Ala Ser 450 His Lys Pro Arg Glu Ser Pro Leu Gly Gly Glu Heu Gly Gly His Ser Glu Gly Ser 450 His Lys Pro Arg Glu Ser Pro Leu Leu Leu Gly Gly Gly His Ser Glu Gly Gly His Ser Glu Ala Ser 450					325					330					335	
Glu Ser Gln Leu Asn Ser Thr Val Val Gly Leu Thr Val 355 Phe Cys Thr Leu Pro Glu Asn Val Gys Asn Ile Val Val 375 Ser Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly Asn 405 Gln Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 Cys Gys Gys Glu Glu Gy Gly Gly Ala Ser Glu Asp Cys 430 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Gly Gly Ala Ser Glu Ala Ser 440 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Gy Gly Ala Ser Glu Ala Ser 450 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly Gly Ala Ser Ser 585	Ωŷ			G13	Arg	Lys		G] u	Cys	Arg	Ala		Lys	His	Ę,	
Glu Ser Gln Leu Asn Ser Thr Val Val Gly Leu Thr Val 355 360 365 Phe Cys Thr Leu Pro Glu Asn Val Cys Asn Ile Val Val 370 Ser Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly 390 Gln Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 Cys Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 430 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 His Lys Pro Arg Glu Ser Pro Leu Gly	•			340					345					350		
355 Phe Cys Ibr Leu Pro Glu Asn Val Cys Asn Ile Val Val 370 Ser Thr Glu Leu Ibr Arg Gln Thr Leu Asp Leu Leu Gly Gln Phe Cys Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 Cys Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser A40 435 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Gly Gly Ala Ser Glu Ala Ser 440 446 447 448 448 449 448 448 449 448 449 448 448	Cys				Leu	Asn		Thr	Val	Val	Gly			Val	Val	
370 Ser Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly 390 395 Gln Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 Cys Lie Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys 425 Gly Ser Gy Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 435 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 His Lys Pro Arg Glu Ser Pro Leu Gly			355					360					365			
370 Ser Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly 390 Gln Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 Cys Lie Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys 420 Cys Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 435 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 475	Ala					Pro				Cys	Asn	11e	Val	Val	A1a	
Ser Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly 395 Gln Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 Cys Ile Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys 420 Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 435 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 Ris Lys Pro Arg Glu Ser Pro Leu Leu Pro Leu Gly His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 475		370					375					380				
396 395 395 395 395 396 396 397 398	Leu					Ę	Arg	Glo	Thr	Leu		Leu		613	Leu	
Gin Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val 405 Cys Ile Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys 420 Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 435 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 455 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 475	385					390					395					
405 Cys Ile Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys 420 Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 435 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 470 470 475	Asn		Phe						Gly	Ala					Leu	
Cys lie Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys 426 Cys Cys Gy Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 435 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 A55 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 475					405					410					415	
420 Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 435 440 440 445 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 475	Leu									A18					Cys	
Gys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser 435 440 445 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 460 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 475				420					425					430		
435 Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser 450 A55 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu 470 475	Cys				CJ.			Gly	613				Ala			
Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser 450 450 His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 470			435					440					445			
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His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly 470 475		450	_				455					460				
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Cys	465					470					475					
	Cys				,											

145	Ala I	-	Pro A		Glu A		. Glu II		Ser Arg	65	Leu Trp	6 0	Leu Leu		Arg Pr		Gly Gly	-	Met Cys	(xi)	(ii)	(a) (b) (c) (a) (c) (a) (c) (c)	INFORMATION
	i.	130	Arg		. Ala		5					50			0					SE	Ö	17	X
	Ser		Pro	115	Lys		Thr Gla		Val		Pro		Phe	35	λla		G1n		Pro	QUE	TEC	SEQUENCE LENGTH TYPE: STRAND TOPOLO	TIO
	Pro		Ile		GLy	100	G1u		Ser		Leu		reī		Álα	20	λla		λla	SEQUENCE	MOLECULAR	O 18 D **	
	Asn		. H.		Val		G1 _D	85	Gly		Ala		Gly		Ser		Ser	ຜ	01u			QUENCE CHARAC LENGTH: 542 TYPE: amino a STRANDEDNESS: TOPOLOGY: lin	FOR S
150	Pro		Pro		Cla		GT p		G1.y	70	Val		Trp		Leu		Αsp		G1 y	SCR	TYPE:	RACTER 2 0 acid SS:	SEQ
Ū	Asp	135	Ala		Gla		Ser		Ala		202	យ	Leu		G1 _u		A1a		Pro	Description:		ACID	Ħ
	Lys	•	C1y	120	Tyr		Ar8		Pro		Leu		Ser	40	Pro		Arg		Ala	Š.	peptide	CHARACTERISTICS 542 mino acid DNESS:	NO:
	Asp		Leu		Val	105	Ser		Leu		Ala		Pro		Ser	25	λrg		Ar 8	SEQ	ę	:S:	2
	Gly		Gln	•	Pro		Lys	90	H:		Val		Ala		Ser		Leu	10	Pro	ä			
155	G1 _y		Pro		G1 _u		Arg		Leu	75	Ile		His		Trp		Thr		Val	D NO:			
-	The	140	Thr		113		G1.y		Gly		neT	60	Pro		λla		Cly		A18	2			
	Pro		Lys	125	Trp		Thr		Arg		Ala		λla	5	Pro		Cly		Gly	:-			
	Asp		Pro		λla	110	ST to		His		Val		Met		Cys	30	C13		GLy				
	Ser		Leu		212		Asp	95	8 1V		Gly		Arg		Thr		Ser	15	Trp				
160	Gly	•	Val		Tyr		5		Ala	88	Leu		Trp		His		Ser		Glu	•			

355	Ala Ser Leu Pro Glu Ser Leu Tyr	340	Pro Ala Pro Thr Met Gly Thr Leu		Met Thr Leu Ala Val Pro Glu Leu	305 . 310	Arg Cys. Gln Ser Ile Leu Ala Lys	290 295	Arg Phe His Val Ala Thr Ser Thr	275 280	Ser Ser Leu Gly Val Thr Thr Phe	. 260	Arg Leu Leu Gly Asp Val Ser Cys	245	Leu Phe Phe Cys Leu Pro Ile Val	225 . 230	Ala Trp Asn Ser Ile Leu Ala Ser	210 215	Asn Leu Ser Val Met Cys Ile Val	195 . 200	Ala Ile Met Leu Leu Ala Leu Val	180	Ile Gln Asn Pro Leu Tyr Pro Val	165	Gln Glu Leu Arg Gly Asn Leu Thr Gly Ala
. 365	Ser Leu Val Met Thr	345 350	Asp Ser Cys Ile Met Lys	330 335	u Leu Leu Trp Gln Leu Ala Gln Glu	315 320	s Leu Ala Val Ile Trp Val Gly Ser	300	r Leu Pro Lys Val Arg Pro Ile Glu	285	Ser Leu Cys Ala Leu Gly Ile Asp	265 270	Arg Ala Val Pro Phe	250 255	Ile Phe Asn Glu Ile Thr Lys Gln	235 240	Leu Ala Leu Trp Asp Phe Leu Val	220	Trp His Ser Tyr Tyr Leu Lys Ser	205	Val Phe Ala Val Gly Ile Val Gly	185 190	Thr Glu Ser Ser Tyr Ser Ala Tyr	170 175	Gly Ala Pro Gly Gln Arg Leu Gln

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.	Pro 400 Ser	8 %	jų.	Phe	11e 480 Cys	Ser	Lys	
[]e [Pro P	415 Phe C	Ser J	Gln	Cys Cys	495 Gly	His	
Ala Arg Met Trp Trp Tyr Phe Cly Cys Tyr Phe Cys Leu Pro Ile Leu 370 380	Phe Thr Val Thr Gys Gin Leu Val Thr Trp Arg Val Arg Gly Pro Pro 385 390 Gly Arg Lys Ser Glu Cys Arg Ala Ser Lys His Glu Gln Cys Glu Ser	415 Gin Leu Asn Ser Thr Val Val Gly Leu Thr Val Val Tyr Ala Phe Cys 420 425 430	Thr Leu Pro Glu Asn Val Cys Asn Ile Val Val Ala Tyr Leu Ser Thr 435	Glu Leu Ibr Arg Gln Thr Leu Asp Leu Leu Gly Leu Ile Asn Gln Phe 450 450	Ser Thr. Phe Phe Lys Gly Ala Ile Thr Pro Val Leu Leu Leu Cys Ile 485 Cys Arg Pro Leu Gly Gla Ala Phe Leu Asp Cys Cys Cys Cys Cys Cys	485 490 495 Cys Glu Glu Gya Gly Gly Ala Ser Glu Ala Ser Ala Asn Gly Ser 500 505 510	Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser Ile Tyr Phe His Lys 515 Pro Arg Glu Ser Pro Pro Leu Eeu Pro Leu Gly Thr Pro Cys 530	
Leu	Arg Gln	Iyr	1yr	116	Leu	Ala	Tyr 525 Pro	
380	Val Glu	Val	A18	Leu 460	Leu	Ala	Ile Thr	2
Phe	Ars 395 His	Va.1	Val	G1 <i>y</i>	Va1 475 Cys	Ser	Ser	
Tyr	Trp Lys	410 Thr	Val	Leu	Pro	490 i Ala	Ser	
Cys	Thr Ser	Leu 425	116	Le	Thr.	. G1u 505	Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser Ile Tyr Phe 515 520 Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly Thr Pro Cys 530 540	
G13	Val	Ę	440	1 AS	a Phe	Ser	1 Val 520 1 Leu	
Phe 375	Leu	Val	Cy3	r Leu 455	¥ 4	y Alb	r Glu o Leu 535	;
Tyr	390 390	7 Val	, Va.1	d E	470 470 9 Gla	5 7 G1;	s Th	
Tri	ខ្លុំ ថ្	405 r Thr	u Ası	5	e Ly	485 a Gly	u Ly	
Tri :	. Se	n Ser 420	. G	r Ar	e Ph	ц Суя 500	s Le	
	Val	1 A81	1 Pro	a C	44 F	. 5	n Lys 515 8 Glu	,
Ar6 370	The The	Let	. Le	450	4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	G	P Asn o Arg	,
A18	Phe 385 Gly	611	Ē	ซี	Ser 465 Cys	Š	A P	

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					9	120	180	240	300	360	420	480	
					GGGCTAAGC	CCAGGAGCAG	GCAGTATGTG	GCCAACCAAG	CAGTGGGCAG	GAACCCCCTG	GCTGGTGGTG	CAGCTACTAC	
			NO: 3:		ATBCGGTGGĊ TGTGGCCCCT GGCTGTCTCT CIIGCIGIGA IIIIGGCTGT GGGGCTAAGG	AGGGTCICTG GGGGTGCCCC CCTGCACCTG GGCAGGCACA GAGCCGAGAC CCAGGAGCAG	CAGAGCCGAI CCAAGAGGGG CACCGAGGAI GAGGAGGCCA AGGGCGIGCA GCACIAIGIG	CCIGAGGAGT GGGCGGAGTA CCCCGGCCC ATTCACCCTG CTGGCTGCA GCCAACCAAG	CCCTTGGIGG CCACCAGCCC TAACCCCGAC AAGGAIGGGG GCACCCCAGA CAGIGGGCAG	GAACTGAGGG GCAAICIGAC AGGGGAGCA GGGCAGAGGC IACAGAICCA GAACGCCGIG	IAICCGGIGA CCGAGAGCIC CIACAGIGCC IAIGCCAICA IGCIICIGGC GCIGGIGGIG	ITIGGGIGG GCAIIGIGGG CAACCIGICG GICAIGIGCA ICGIGIGGCA CAGCIACIAC	
. 3	ICS:		SEQ ID NO:		CITGCIGIGA	GGCAGGCACA	GAGGAGGCCA	ATTCACCCTG	AAGGATGGGG	GGGCAGAGGC	TATGCCATCA	GTCATGTGCA	
INFORMATION FOR SEQ ID NO:	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1443 (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear	(ii) MOLECULAR TYPE: CDNA	(xi) SEQUENCE DESCRIPTION:		GCCTCTCTCT	CCTGCACCTG	CACCGAGGAT	ວວວອອວວວວວ	TAACCCCGAC	AGGGGCACCA	CIACAGIGCC	CAACCIGICG	
TION FOR	QUENCE CHARACTER LENGTH: 1443 TYPE: nucleic ac. STRANDEDNESS: doi TOPOLOGY: linear	LECULAR T	QUENCE DE		TGTGGCCCCT	GGGGTGCCCC	CCAAGAGGG	GGGCGGAGTA	CCACCAGCCC	GCAATCTGAC	CCGAGAGCTC	GCATTGTGG	
INFORMA	(i) SEQ (A) L (B) T (C) S (C) S	(ii) MO	(xi) SE		ATGCGGTGGĊ	AGGGTCTCTG	CAGAGCCGAT	CCTGAGGAGT	CCCTTGGTGG	GAACTGAGGG	TATCCGGTGA	TITGCGGTGG	
	ug.	5		15	\$	R		52		30			38

INFORMATION FOR SEQ ID NO: 4

	CIGAAGAGCG CCIGGAACIC CAICCIIGCC AGCCIGGCCC ICIGGGAIIT ICIGGICCIC	540	
υ	ITITICTGCC TCCCTAITGI CAICTICAAC GAGATCACCA AGCAGAGGCT ACTGGGTGAC	600	
	GITICTIGIC GIGCCGIGCC CIICAIGGAG GICICCICIC IGGGAGICAC GACIIICAGC	660	
	CICIGIGCCC IGGGCATIGA CCGCITCCAC GIGGCCACCA GCACCCIGGC CAAGGIGAGG	720	
70	CCCATCGAGC GGTGCCAATC CATCCTGGCC AAGITGGCTG ICATCTGGGT GGGCTCCATG	780	***
	ACCCTGCCTG TGCCTGACCT CCTGCTGTGG CAGCTGGCAC AGGAGGCTGC CCCCACCATG	840	
	GGCACCCIGG ACTCATGCAI CAIGAAACCC ICAGCCAGCC IGGCCGAGIC CCIGIAIICA	900 ·	-
	CIGGIGAIGA CCIACCAGAA CGCCCGCAIG IGGIGGIACI IIGGCIGCIA CIICIGCCIG	980	
	CCCATCCTCT TCACAGTCAC CTGCCAGCTG GTGACATGGC GGGTGCGAGG CCCTCCAGGG	1020	
20	AGGAAGTCAG AGTGCAGGGC CAGGAAGCAC GAGCAGTGTG AGAGCCAGCT CAACAGCACC	1080	B.
	GIGGIGGGC IGACCGIGGI CIACGCCIIC IGCACCCICC CAGAGAACGI CIGCAACAIC	1140	
25	GIGGIGGCCI ACCICICCAC CGAGCIGACC CGCCAGACCC IGGACCICCI GGGCCICAIC	1200	Po.
	AACCAGITCI CCACCIICII CAAGGGCGGC ATCACCCCAG IGCIGCICCI IIGCAICIGC	1260	
:	AGGCCGCTGG GCCAGGCCTT CCTGGACTGC TGCTGCTGCT GCTGCTGTGA GGAGTGCGGC	1320	
8	GGGGCTTCGG AGGCCTCTGC TGCCAATGGG TCGGACAACA AGCTCAAGAC CGAGGTGTCC	1380	
	TOTTCCATCT ACTTCCACAA GCCCAGGGAG TCACCCCCAC TCCTGCCCCT GGGCACACCT	1440	
35	TGC	1443	6.5

GCCATGCGGT	AGCTCTTGGG	AGTGATGCCA	ATGTGTCCAG	(xi) SE	(ii) MC	(i) SEQ (A) L (B) I (C) S (C) I
CCCVICCOLI COCLICIOCO COLOCOLOLO CLICOLOCO LOVILLOCO LOS LOCALOCO	AGCICTIGGG CCCCCIGIAC TCACCIGGIC ITCCTGGGCT GGCIGTCICC TGCTCAICCA	AGTGATGCCA GACGCCTGAC IGGAGGCGGA ICCAGCCGGC CAGCIGCCIC ICIGGAGCCC	ATGIGICCAG CAGAGGGCCC IGCCCGGCCI GIGGGCGAG GCIGGGAGGG AGGGCAGGG	(xi) SEQUENCE DESCRIPTION:	(11) MOLECULAR TYPE: cDNA	A) LENGTH: 1626 B) TYPE: nucleic acid C) STEANDEDWESS: double D) TOPOLOGY: linear
ccreecrere	TCACCIGCIC	TGGAGGCGGA	IGCCCGGCCI	SCRIPTION:	YPE: CDNA	RACTERISTI 26 eic acid ess: double
ICICITECIE	TICCIGGGCT	TCCAGCCGGC	GIGGCCGGAG	SEQ		. CS:
IGATITITGGC	GCCIGICICC	CAGCIGCCIC	CCIGGGAGGG	ID NO: 4:		
ALDSDSDSIDI.	TGCTCATCCA	TCTGGAGCCC	AGGGCAGGCG			

GCAGGGTCT (CIGGGGGTGC (CIGGGGTGC CCCCTGCAC CIGGGCAGGC		ACAGAGCCGA GA	GACCCAGGAG	300
CAGCAGAGCC	GATCCAAGAG (פפכביככפים	GATCCAAGAG GGGCACCGAG GATGAGGAGG CCAAGGGCGT		GCAGCAGTAT	360
STGCCTGAGG	AGTGGGGGGA GTACCCCCGG	GTACCCCCGG (CCCATTCACC	כופכופככנו פ	GCAGCCAACC	420
AAGCCCTTGG	TGGCCACCAG	TGGCCACCAG CCCTAACCCC GACAAGGATG	GACAAGGATG	GGGCACCC AGACAGTGG	GACAGTGGG	480
CAGGAACTGA	GGGCAATCT GACAGGGGA	GACAGGGGCA	CCAGGGCAGA GGCTACAGAT		CCAGAACCCC	240
CTGTATCCGG	TGACCGAGAG	CTCCTACAGT	GCCTATGCCA	TCATGCTTCT G	GCCCTGGTG	900
GIGITIGGGG IGGGCATIGI	TGGGCATTGT	GGGCAACCTG	TCGGTCATGT	GCAICGIGIG G	GCACAGCTAC	099
TACCTGAAGA	GCGCCIGGAA CICCAICCII		GCCAGCCTGG	CCCTCTGGGA 1	TITICIGGIC	720
CTCTTTTCT	GCCTCCCTAT	TGTCATCTTC	GCCICCCIAI IGICAICIIC AACGAGAICA	CCAAGCAGAG	GCTACTGGGT	780
GACGITICIL	GTCGIGCCGI	ercereccer eccercate	GAGGICICCT	CICICCCAGI (CACGACTITC	840
AGCCTCTGTG	CCCTGGGCAT	TGACCGCTIC	CACGTGGCCA	CCAGCACCCT	GCCCAAGGTG	900
AGGCCCATCG	AGCGGTGCCA	ATCCATCCTG	AGCGGTGCCA ATCCATCCTG GCCAAGTTGG	CTGTCATCTG	GGTGGGCTCC	960
ATGACGCTGG	CTGTGCCTGA	GCTCCTGCTG	TGGCAGCTGG	CACAGGAGCC	TGCCCCCACC	1020
ATGGGCACCC	TGGACTCATG	CATCATGAAA	CCCTCAGCCA	IGGACICAIG CAICAIGAAA CCCICAGCCA GCCIGCCGA GICCCIGIAI	GTCCCTGTAT	1080
TCACTGGTGA		GAACGCCCC	TGACCTACCA GAACGCCCCC ATGTGGTGGT	ACTITEGETE	CTACTTCTCC	1140
CIGCCCAICC	FCTTCACAGT	CACCTGCCAG	CACCIGCCAG CIGGIGACAT	GGGGGGTGCG	AGGCCCTCCA	1200
GGGAGGAAGT	CAGAGTGCAG	GGCCAGCAAG	CACGAGCAGT	GTGAGAGCCA	GCTCAACAGC	1260
ACCGIGGIGG	GCCIGACCGI		GGICIACGCC TICIGCACCC	TCCCAGAGAA	CGTCTGCAAC	1320
ATCGTGGTGG	CCTACCTCTC	CACCGAGCTG	CACCGAGCTG ACCCGCCAGA	CCCIGGACCT	CCTGGGCCTC	1380
ATCAACCAGT	TCTCCACCIT		CITCAAGGGC GCCATCACCC	CAGTGCTGCT	CCTTTGCATC	1440
TGCAGGCCGC	recadecede regeceadee	CTICCICCAC	CTICCIGGAC IGCIGCIGCI	GCTGCTGCTG	TGAGGAGTGC	1500
GCCGCCCTT		C TGCTGCCAA1	GGGTCGGACA	CGGAGGCCIC IGCIGCCAAI GGGICGGACA ACAAGCICAA GACCGAGGIG	GACCGAGGTG	1560
TCCTCTTCCA		TCTACTICCA CAAGCCCAGG	GAGTCACCCC	CACTCCTGCC	CCTGGGCACA	1620
CCTTGC						1628

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CAAGAG GGGCACCGAG GATGAGGAGG CCAAGGGCGT GCAGCAGTAT	TCAGGAGG	CCAAGGGCGT G	CAGCAGTAT	360		(i)
GGCGGA GIACCCCCGG CCCAIICACC CIGCIGGCCI GCAGCCAACC	CATTCACC	CIGCIGGCCI 0	CAGCCAACC	420		۔ ت
CACCAG CCCIAACCCC GACAAGGAIG GGGGCACCCC AGACAGIGGG	ACAAGGATG	eeeccycccc /	GACAGTGGG	480		
CAATOT GACAGGGCA CCAGGGCAGA GGCTACAGAT CCAGAACCCC	CAGGGCAGA	GCTACAGAT	CAGAACCC	540	01	(ii
CGAGAG CICCIACAGI GCCIAIGCCA ICAIGCIICI GGCGCIGGIG	CCTATGCCA	TCATGCTTCT (эссстеста	009		(xi
CATICI GGGCAACCIG ICGGICAIGI GCAICGIGIG GCACAGCIAC	CGGTCATGT	GCATCGTGTG (CACAGCTAC	099	15	
CUGGAA CICCAICCII GCCAGCCIGG CCCICIGGGA IIIICIGGIC	CCAGCCTGG	CCCTCTGGGA	TITICICATC	720	ì	
ICCCIAI IGICAICIIC AACGAGAICA CCAAGGAGAG GCIACIGGGI	ACGAGATCA	CCAAGCAGAG	SCTACTGGGT .	780		TGCAATC
DIECCOT GCCCTICATG GAGGICICCT CICIGGGAGI CACGACITIC	AGGICICCI	CICIGGGAGI	CACGACTITC	840	20	CIGAGCI
TEGGCAT TGACCGCTTC CACGTGGCCA CCAGCACCCT GCCCAAGGTG	ACGTGGCCA	CCAGCACCCT	GCCCAAGGTG	008	3	CATGCAL
GGTGCCA ATCCATCCTG GCCAAGTTGG CTGTCATCTG GGTGGGCTCC	CCAAGTTGG	CTGTCATCTG	GOTGGGCICC	980	Y 52	ACCAGAA
TGCCTGA GCTCCTGCTG TGGCAGCTGG CACAGGAGCC TGCCCCCACC	GGCAGCTGG	CACAGGAGCC		1020	3	CAGICAC
ACTCATG CATCATGAAA CCCTCAGCCA GCCTGCCGGA GTCCCTGTAT	CCTCAGCCA	GCCTGCCCGA		1080		GCAGGG
CCIACCA GAACGCCCGC AIGIGGIGGI ACTIIGGCIG CIACTICIGC	VICTGCTGGT	ACTITGGCTG		1140	e e	CCGTGGI
TCACAGI CACCIGGCAG CIGGIGACAI GGCGGGIGCG AGGCCCIGCA	CIGGIGACAI	GGCGGGTGCG		1200	Ö	CCLLIL
AGTECAG GECCACCAAG CACGAGCAGT GTGAGAGCCA GCTCAACAGC	CACGAGCAGT	GTGAGAGCCA		1260	35	
TGACCGT GGTCTACGCC TTCTGCACCC TCCCAGAGAA CGTCTGCAAC	TCTGCACCC	TCCCAGAGAA	CGTCTGCAAC	1320		
ACCIGIC CACCGAGCIG ACCCGCAGA CCCIGGACCI CCIGGGCCIC	ACCCCCCAGA	CCCIGGACCI	CCTGGGCCTC	1380	ş	
TCCACCII CIICAAGGGC GCCAICACCC CAGIGCIGCI CCIIIGCAIC	CCATCACC	CAGTGCTGCT	CCTTTGCATC	1440	:	
SECCAGGE CITECIGEAC IGCIGCIGCI GCIGCIGCIG IGAGGAGIGE	тестестест	. מכומכומכום	TGAGGAGTGC	1500		
SAGGCCIC IGCIGCCAAI GGGICGGACA ACAAGCICAA GACCGAGGIG	GGGTCGGACA	ACAAGCICAA	GACCGAGGTG	1560	3	
TACTICCA CAAGCCCAGG GAGICACCCC CACICCIGCC CCIGGGCACA	GAGTCACCC	: CACTCCTGCC	CCTGGGCACA	1620		
				1628	99	

300 360 420 426

Va.	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 426 (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear
9	(ii) MOLECULAR TYPE: CDNA
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
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	IGCAATCCAT CCIGGCCAAG IIGGCIGICA ICIGGGIGGG CIGCAIGACG CIGGCIGIGG
8	CIGAGCICCI GCIGIGGCAG CIGGCACAGG AGCCIGCCC CACCAIGGGC ACCCIGGACI
	CAIGGAICAI GAAACCCICA GCCAGCCIGC CCGAGICCCI GIAIICACIG GIGAIGACCI
	ACCAGAACGC CCGCAIGIGG IGGIACTIIG GCIGCIACII CIGCCIGCCC AICCICIICA
SS	CAGTCACCTG CCAGCTGGTG ACATGGCGGG TGCGAGGCCC TCCAGGGAGG AAGTCAGAGT
	GCAGGGCCAG CAAGCACGAG CAGTGTGAGA GCCAGGTGAA CAGCACGGTG GTGGGCGTGA
99	CCGTGGICTA CGGCITITIG CAACCIICCA GAGAACGIII GCAACAICGI GGIGGGCITA

INFORMATION FOR SEQ ID NO: 6

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 248
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: CDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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8 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7: AAGIIGGCIG TCATCIGGGI GGGCIC

26

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(ii) MOLECULAR TYPE: synthetic DNA

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear (ii) MOLECULAR TYPE: synthetic DNA INFORMATION FOR SEQ ID NO: 8

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8: TGAGCTCCTG CTGTGGCAGC TGGCACAG 28

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INFORMATION FOR SEQ ID NO: 9

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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CAIGCGGGGG TICTGGTAGG TCATCAC

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INFORMATION FOR SEQ ID NO: 7

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

જ 53 3 8 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12: (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11: (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10: 21 21 28 (11) MOLECULAR TYPE: synthetic DNA ATGACAATAG GGAGGCAGAA AAAGAGG (ii) MOLECULAR TYPE: synthetic DNA (11) MOLECULAR TYPE: SYNTHETIC DNA ATGAAGGGCA CGCCACGACA AGAAACG GAAGAGGATG GGCAGGCAGA AGTAGCAG (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear INFORMATION FOR SEQ ID NO: 12 INFORMATION FOR SEQ ID NO: 11 (i) SEQUENCE CHARACTERISTICS: INFORMATION FOR SEQ ID NO: 10 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27(B) TYPE: nucleic acid(C) STRANDEDNESS: single (A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear (D) TOPOLOGY: linear

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INFORMATION FOR SEQ ID NO: 13

(1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 33 (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GICGACGAGA TGTGTGAGGG CAGCAAAGAG TGC

33

INFORMATION FOR SEQ ID NO: 14

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

82 TACTEGEGCC TCAGCAAGGT GTGCCCAG

INFORMATION FOR SEQ ID NO: 15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single (D) TOPOLOGY: linear

(11) MOLECULAR TYPE: synthetic DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

32 GICGACIGGC IGICICCIGG ICAICCAGCC AI

Claims 22

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A G-protein coupled receptor protein which comprises the same or substantially the same arnino acid sequence
as that represented by SEQ ID NO: 1, or a variant of the arnino acid sequence having a deletion, addition or

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substitution of 1 to 30 amino acids, or its salt

'n or substitution of 1 to 30 amino acids, or its salt. sequence as that represented by SEQ ID NO: 2, or a variant of the amino acid sequence having a deletion, addition The G-protein coupled receptor protein of claim 1 which comprises the same or substantially the same amino acid

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- ယ A partial peptide of the G-protein coupled receptor protein of claim 1 or its salt
- 4 An isolated DNA comprising DNA having a nucleotide sequence encoding the G-protein coupled receptor protein

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- òυ The isolated DNA of claim 4 having the nucleotide sequence represented by SEQ ID NO: 3.
- The isolated DNA of claim 4 having the nucleotide sequence represented by SEQ ID NO: 4.

A recombinant vector comprising the DNA of claim 4.

- A transformant comprising the recombinant vector of claim 7.
- 20 9 The transformant of claim 8 which is Escherichia coli HB101/pHEBF2 (FERM BP-5724).
- 10. A process for preparing the G-protein coupled receptor protein of claim 1 or its salt which comprises cultivating the transformant of claim 8 to form the G-protein coupled receptor protein and recovering it.
- 25 11. A method for determining a ligand to the G-protein coupled receptor protein of claim 1 or its salt which comprises bringing the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or its salt into contact with a test compound.
- 12. A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of claim 1 or its salt, or their salts which comprises comparing

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- (i) ligand binding upon bringing the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide
- of claim 3 or its salt into contact with the ligand, and
 (ii) that upon bringing the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or its salt into contact with the ligand and a test compound.

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13. The screening method according to claim 12, wherein ligand binding is compared by means of an amount of a or cell stimulation activities mediated by the G-protein coupled receptor protein of claim 1 to the G-protein coupled receptor protein of claim 1 expressed on a cell membrane of the transformant of claim 8; fraction of cells containing the G-protein coupled receptor protein of claim 1; an amount of a labeled ligand bound 3 or its salt; an amount of a labeled ligand bound to cells containing the G-coupled receptor protein or a membrane labeled ligand bound to the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim

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- Ġ 14. A kit for screening for compounds which after binding of a ligand to the G-protein coupled receptor protein of claim claim 1 or its salt or the partial peptide of claim 3 or its salt. or its salt, or their salts which comprises as an essential component the G-protein coupled receptor protein of
- 8 15. The kit of claim 14, wherein the component is in the form of cells containing the G-protein coupled receptor protein of claim 1 or its salt or a cell membrane fraction of cells containing the G-protein coupled receptor protein of claim
- A compound which alters ligand binding to the G-protein coupled receptor protein of claim 1 or its salt obtained by the screening method of claim 12 or the kit of claim 14

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દ 17. An antibody against the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or

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18. A method for determining the G-protein coupled receptor protein of claim 1, or its salt or the partial peptide of claim

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3 or its salt in a specimen which comprises bringing the antibody of claim 17 into contact with the G-protein coupled receptor protein of claim 1, the partial peptide of claim 3 or a salt thereof in the specimen.

- 19. A pharmaceutical composition comprising as an effective component the G-protein coupled receptor protein of claim 1, or its salt or the partial peptide of claim 3 or its sall, and a pharmaceutically acceptable carrier or diluent
- 20. A pharmaceutical composition comprising as an effective component the compound of claim 16 or its salt, and a pharmaceutically acceptable carrier or diluent

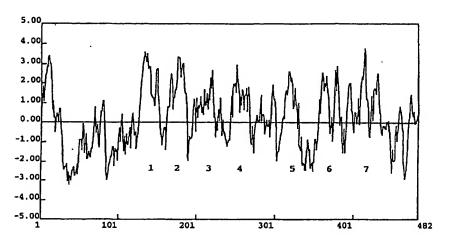
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	ATCCTAATAGGACTCACTATAGGCTCCAGCGCCCCCCCCGGGGGTCTCTCGAGGCGCA	3 -	
F He	TCTODGTTGTGGGCCTTCTTCTTGTATCAACTATGTGGCAAGTGACTGAC	120	
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~ ~	AGACCTAGTNAGTGTTCAGTAAGTGACAGTGACGGTTATTGCTGAGTCTTGAATGCAAG	240 1	
	AGCTOCCTTNGANTCAGGAGACTTGGGCCCAGTTCCCANTCTGCCCCACCTCCTGTGT	300	
8-	CACCCTAGGCAGGCACATTTCCTCCCTAGTTTCAGGGGCCTGAAAGCAGTGCCCTCTTA	360	
್ಡ -	GOCCECACACCTCACATACTOTACOCCTCACAACOOCCTCCTOCAGCACCACATGT	1 1	
ដ្ឋក	GTGAGGGCAGCAAAGAGTGCTATGTGTCAGCAGAGGGCCTGCCCGCCTGTGGCCGGA	480	
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1261	1 TCTCTOGGAGTCACGACTTTCAGGCTCTGTOGGCTGGGGCATGAGGGCTTCCACGTGGCC SectenGlyValthcTheSectenCygAlatevGlyLeAgpAcgPheHisValAla	222	
122	1 MCMCMCCATCOCOMBATCHCACCATCACCATCCATCCATCCTCCCAAAATTG 1 ThrSetThrLeuProlysValAtgProlleGluArgCyRGlnSet1leLeuAlleLysLeu	222	

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Fig.

2 4	2041 CCACTCCTGCCCCTGGCCACCTTGCTGAGGCCCCAGTA	2
, 4 0, 4	81 AACAAGCTCAAGACCGAGGTGTCTTCTACTACTTCCACAAGCCCAGGGAGTCACCC 43 AanlysleulysthagluValSetSet1letyrbhehlstysbroktgGluSetPto	1981
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25.	61 COMORCIOCTOCTTIOCATCTOCAGOSCOCTOSOCOAGGCTTCCTGGACTOCTGCT 413 ProvalLeuleuleuCyslleCyskrgFroleuGlyGinAlaPheleukspCysCysCys	1861
186		1801
3 68	41 CTCCCAGAGAACCTCTCCAACATCGTGGCCTACCTCTCCACCGCAGGTGACCTGACCGCAG 313 LeuproglwasnValcyaAsnIleValValalaTyrLeuSerThrGluLeuThrArgGln	1741
471 CE	1 TOTALANGCCHACTCHACACACCOTOGOGOCTCHACCGTTOTCTACGCCTTCTCCACC C CysCluserGlileuasnSerfhrValValQilyleufhrValValValYrhlaPheCysThr	1681
35	1 TOCOGGETICGAGOCCCTCCAGGGAGAGTCAGAGTCCAGGGCCAGCAGCAGAGAGAG	1621
333	1 TACTTIGGCTGCTACTTCTGCCTGCCCATCTTCACAGTCACCTGCCAGTGGTGACAGTGGTGAGAGTGAGAGAGA	1361
31.	i ACCTICCCCGAGTCCCTGTATTCACTGGTCATCACCTACCAGAACGCCCCCATGTGGTGG S SerLeuProGluSerLeuTyrSerLeuValMetThrTyTGlahsnAlaArgHetTrPfrP	233
23,2	. GCACHGGAGCTIGCCCCCACCATGGGCACCTGGACTCATGCATGCATGCACCCTCAGCC i AlaGlaGluProAlaProThrHetGlyThrLeuAspSercysIlehetLysProSerAla	441
7,7	GCTGTCATCTGGGTGGCTCCAleator is a control of the con	25 25



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61 TOTOGOTTOTOGOCTTOCTCTOGCTTOTATCAACTATUTGGCAAGTGACTGACCTCTAC 1 апестализация стементальности в в составления в составления в 1

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181 AGCACCTAGTAAGTGTTCAGTAAGTGACCAGTTATTGCTGAGTCTTGAATGGAGG

361 GOGCCCCHEAGCCTGACATCCTGTAGGCCCTGAGAAGGCGCGTGGTGGAGGACGAGATGT 301 CACCCTAGGCAGGCAGATTTCCTCCCTAGTTTCAGGGGCCTGAAGGAGGAGGCCCTCTTA

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1141 GCCTCTGGGATTTTCTGCTCTTTTTCTGCTCCTGTTGTCATCTTCAACGAGATC
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1121 ACEAGUACOCOCCUMGOROAGACUNTCOAGGGGTGCUMTCANCTGGCAAGTTG 113 ThrSetThrLeutroLynValletgtroIleGlwlegCysGloSetIleLeullalysLeu 1201 ACCAACHAGACHACTACATULCATTICTTOTICCICCCGTGCCCTTCATGGAGGTCTCC 253 ThruyeGhArquedLeuGlyAspValSetCysArgAlaValProPheleacGluValSet

1120 293 1180

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Fig. 4

Fig. 3 (continued)

1920 493 513 513 1740 £33 \$ £ 1680 413 333 1921 TECTGCTGCTGTGAGGAGTGCGGGGGGGGGTTGGGAGGCCTCTGCTGCCAATGSGTGGGAG 493 CysCysCysCysGluGlaCysGlyGlyAlaSerGluAlaSerAlaAlaAsmGlySerAsp 1741 CTCCCAGAGAACGTCTGCAACATCGTGGCTBACCTCTCCACGAGCTGACGCAG 433 LeuprogluAsnValcysAsmileValValAlafyrLeuSerThrGluLeuThrArgGln 1801 ACCCTICANCTICATIONACHACASTICACCACTTCTICAAGGGGGCCATACC 453 Thrieubspieuleudlyleulleasmóluphséerfhribhsphelysólyalallefhr 1441 GCMCMGGAGCCTGCCCCCCACAGGGGCACCCTGGACTCATGCATCATGCATCATGCGTCAGCC 333 AlaGinGiuProAlaProThrMetGlyfftrLeukspSerCys1leMetLysProSerAla 1501 MOCCIGCOGNOTOCTOTMITCACTGOTGATGACTACCAGAAACGCOCCANGIGGTGG 1561 TACTTIGGCTGCTACTTCTGCCTGCCTCCTTCACAGTCACCTGCCACCTGCTGCACA 373 TyzPheGlyCysfyrPheCysLeubrolleLeubhefhrValfhrCysGlnLeuvalfhr

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1981 NACHAGTICHAGACCAGATGTCCTCTACATTCACTAGCCAGGGAGTCACCC 513 AsaLystealysThrolwValSerSerSerIleTyr?heidsystyschegdwserPro

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2041 CCACTCCTGCCCCTGGGCACACCTTGCTGAGGCCCCAGTA 533 ProleuleubrolewGlyThrProCys***

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· Kidney
· Pancreas
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 · Thymus
 · Prostate
 · Testis
 · Uterus
 · Small Intestine
 · Colon
 · Peripheral Blood
 Leukocyte
- · Gerebellum
 · Cerebral Cortex
 · Meduilla
 · Occipital Pole
 · Frontal Lobe
 · Temporal Lobe
 · Putamen
 · Spinal Cord

- · Amygdala
 · Caudate Nucleus
 · Corpus Callosum
 · Hippocampus
 · Whole Brain
 · Substantia Nigra
 · Subthalamic Nucleus
 · Thalamus

